BIO-TEK®

KC4

User's Guide

for KC4 Signature and KC4 v3.4

User's Guide to

KC4™ v3.4 and KC4™ *Signature*

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Document Revision

Rev / Date	Changes
A / March, 2001	First Issue.
B / December, 2002	Updated entire manual to correlate with new licensing options, KC4 v3.1 and KC4 *Signature*, and their related new and modified features.
C / September, 2003	Changed KC4 “v3.1” to “v3.3” Added instructions for creating Read & Dispense protocols for SynergyHTTR w/Injectors. Added instructions for configuring and controlling the Dispense Module. Added instructions for using the Database Upgrade Utility.

D / July, 2004	Changed KC4 “v3.3” to “v3.4”
	Added instructions for using KC4 to control the new Clarity™ Microplate Luminometer.
	Added instructions for using the new Multi-Mode detection method for the Synergy™ HT line of instruments.
	Added information for handling an unexpected termination of a kinetic read.
	Updated the system requirements for using PowerReports to include Microsoft® Office XP and Office 2003.
	Updated information on COM port selection (now supports up to nine ports).
	Additional cosmetic changes throughout.
E / March, 2005	Revision D1 one-page insert removed; revision letter changed as a result.

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Introduction

KC4 Software

Bio-Tek's **KC4™** software provides rapid, flexible, and powerful assay analysis with a multitude of applications. The user interface is simple and uncluttered, and provides a quick, step-by-step setup and read process for even the most complex assays.

Applications include:

- Research
- DNA quantitation
- Fluorescence assays
- Bio-Industrial
- Pharmaceutical
- Development

Interfaces with most Bio-Tek autoreaders:

Clarity™	FLx800™	PowerWaveX 340
EL800™	PowerWave™	PowerWaveX Select
ELx800™	PowerWave 340	PowerWave XS
EL808™	PowerWave HT	Synergy™ HT
ELx808™	PowerWave HT 340	µQuant™
FL600™	PowerWaveX™	

Extensive and complete analysis of your laboratory applications:

- Fluorescence and absorbance ELISA
- Spectral scanning from 200-999 nm with the µQuant and all PowerWave, PowerWaveX, and Synergy HT models
- Endpoint assays
- Kinetic assays
- Endotoxin analysis
- Cytotoxicity and titer assays

Defines and controls all reading protocols:

- Selection of filters/wavelengths
- Shaking parameters
- Incubation
- Flexible kinetic options
- Read/Dispense options
- Optional manual entry of data
- Reader diagnostics testing and archiving

Customized assay development:

- **Graphical input** for template creation with auto-replication and fill, and customized well ID text and colors
- **Quick entry** of standard concentrations and sample dilutions with auto-increment and auto-factor
- **Control** and **assay validations** may be created for each assay
- **Multiple sample curves** displayed and analyzed simultaneously, including interpolations
- Powerful **multi-level transformation formulas** allow manipulation of raw data, transformed data, curve-fit results, or statistical calculations at any point in the analysis
- KC4's **zoom feature** allows detailed information observed for each well, including the ability to highlight specific kinetic reaction wells (absorbance and fluorescence)
- **Sample IDs** may be entered manually or imported from a file
- **Extensive curve-fitting capabilities,** including Linear or Log axis definition, Point-to-Point, Linear, Polynomial, Cubic Spline, 4-P, and interpolation and graphic editing features

Customized display and printed reports:

- Quick access to all available **results data** including raw measurement values, transformed values, kinetic calculations, concentrations, plate layout, and statistical data on controls, standards, and samples
- **Masking** and recalculation capability for authorized users
- **Kinetic options:** Real-time display of kinetic reactions, individual well zoom, Mean V, Max V, time at Max V, Min/Max OD, Integral (area under the curve), and Onset calculations
- **Screening options:** Definition of cutoffs by algebraic formulae, and positive, negative, etc., range calculations
- **Validation options:** Formula to validate the Mean, Standard Deviation, or CV of controls
- **Printed reports** are completely customizable and can include results data sets in matrix or column format; standard, kinetic, or dilution curves; sample list; audit trail; protocol description; custom header; and page breaks

- For users with Microsoft® Word/Excel 2000 (or greater) and Windows 98/NT/2000/XP, **PowerReports** links KC4 with Excel and Word, providing a powerful format for creating custom publication-quality report and export documents
- Reports can be **previewed** before printing

Data exporting:

- Data may be **exported** automatically for use with other programs including Excel™, Lotus™, and Paradox™ for further manipulation or report merging
- Exporting is not limited to just raw data; **all calculated results** may be exported

KC4 *Signature* complies with FDA's Electronic Signatures rule, 21 CFR Part 11:

- **System Administrator.** A site-designated System Administrator creates and maintains Standard User (low level) and Power User (high level) accounts, and specifies which KC4 functions shall be protected from use by Standard Users. The System Administrator sets special password and login characteristics including minimum password length, password aging, and idle session time-out.
- **Support for multiple users.** Each user is identified by a unique combination of User ID and encrypted password. Users must log into KC4 with these identifiers to gain access.
- **Time-stamped audit trails.** Activities such as user login/logout, protocol and data file creation and modification, and plate reading are permanently logged in a secure database.
- **Embedded signatures.** Authorized users can electronically sign protocol and data files. Electronic signatures are permanent and remain a part of the overall data record for the life of that record.
- **Secure record storage.** KC4 proprietary files (.prt, .pla, and .glb) are stored in a secure shared-access database. Activities performed on files within this database (such as rename, move, copy, overwrite, and delete) are performed within the KC4 environment, and every change is tracked in an audit trail. In addition, the System Administrator can configure the system so that these activities may only be performed by Power Users.
- **Protected functions.** The System Administrator can protect a variety of functions from use by Standard Users. These functions include the deletion, rename, modification, and overwrite of various record types.

Technical Support

KC4 is backed by a superior support staff. If the software fails to function perfectly, please contact the Technical Assistance Center (TAC) and provide the following information:

- The KC4 software version and revision numbers (such as Version # 3.4, Rev # x) found under the **About** menu.
- The license type ('v3.4' or '*Signature*').
- The product serial number.
- The specific steps which reproduce your problem.
- Any error messages displayed on the screen.
- A daytime phone number and fax number (if available).
- Your name and company information.

Phone Support

Contact the Technical Assistance Center between 8:30 AM and 5:30 PM Eastern Standard Time (EST), and/or Customer Service between 8:00 AM and 5:30 PM EST, Monday through Friday, excluding holidays.

Bio-Tek Instruments' Main Number:	(802) 655-4040
Technical Assistance Center:	(800) 242-4685 (toll free in the U.S.)
	(802) 655-4740 (outside the U.S.)
European Coordination Center:	+49 (0) 7136 9680

Facsimile Support

Send a fax with your questions or requests for help 24 hours a day to:

Technical Assistance Center:	(802) 655-3399
European Coordination Center:	+49 (0) 7136 968 111

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Electronic Communication

Send E-Mail or find us on the Web:

E-Mail: tac@biotek.com

Web: http://www.biotek.com

To visit the our website from within KC4, select **About|Bio-Tek Home Page.**

Installation and Configuration

Important!

There is a certain sequence of events that *must* be followed to ensure that KC4 is properly installed and configured. This chapter describes the essential installation and configuration tasks.

Review the installation options provided on the following pages and choose the one required by your site to determine which tasks must be performed. Turn to the appropriate page number for detailed instructions on each task.

Special note for Clarity™ Microplate Luminometer users: The Clarity PC software must also be installed on the PC(s). Install Clarity *before* performing the step "Connect reader(s) to the computer and establish communication."

KC4 v3.4 – Single PC with one or more users

Step	Task	Page
1	Ensure that the computer meets the minimum system requirements	9
2	Install KC4 and restart the computer	11
3	Start KC4 and log in as the System Administrator	18
4	(Optional) Change the System Administrator's password	21
5	(Optional) Copy KCShared.mdb to a secure network location	22
6	(Required if step 5 was performed) Configure KC4 to use the KCShared.mdb file on the network	23
7	Connect reader(s) to the computer and establish communication	25

KC4 v3.4 – Multiple PCs in a network environment

Step	Task	Page
1	Ensure that all computers meet the minimum system requirements	9
2	Install KC4 on one computer and restart the computer	11
3	Start KC4 and log in as the System Administrator	18
4	Change the System Administrator's password	21
5	Copy KCShared.mdb to a secure network location	22
6	Configure KC4 to use the KCShared.mdb file on the network	23
7	Connect reader(s) to the computer and establish communication	25
8	Repeat steps 2, 3, 6, and 7 for the remaining computers	

Special note for Clarity™ Microplate Luminometer users: The Clarity PC software must also be installed on the PC(s). Install Clarity *before* performing the step "Connect reader(s) to the computer and establish communication."

KC4 *Signature* – Single PC with one or more users

Step	Task	Page
1	Appoint a System Administrator	8
2	Ensure that the computer meets the minimum system requirements	9
3	Install KC4 and restart the computer	11
4	Start KC4 and log in as the System Administrator	18
5	Change the System Administrator's password	21
6	Copy KCShared.mdb to a secure network location	22
7	Configure KC4 to use the KCShared.mdb file on the network	23
8	Create one or more new user accounts	24
9	Connect reader(s) to the computer and establish communication	25

KC4 *Signature* – Multiple PCs in a network environment

Step	Task	Page
1	Appoint a System Administrator	8
2	Ensure that all computers meet the minimum system requirements	9
3	Install KC4 on one computer and restart the computer	11
4	Start KC4 and log in as the System Administrator	18
5	Change the System Administrator's password	21
6	Copy KCShared.mdb to a secure network location	22
7	Configure KC4 to use the KCShared.mdb file on the network	23
8	Create new user accounts	24
9	Connect reader(s) to the computer and establish communication	25
10	Repeat steps 3, 4, 7, and 9 for the remaining computers	

Appointing a System Administrator

☑ **KC4 *Signature***

Before installing the software, one individual at the site must be appointed as the **KC4 System Administrator.** The System Administrator is typically responsible for the following:

- Determining which computers will have KC4 installed on them
- Determining which users will have access to certain KC4 functions
- Installing KC4
- Creating and maintaining user accounts
- Customizing security settings
- Reviewing records
- Establishing a procedure and schedule for record retention and archival

Administrator Requirements

The FDA's Electronic Signatures Rule (21 CFR Part 11) contains requirements that sites must meet in order to be in compliance. The KC4 *Signature* System Administrator should be cognizant of the following:

- **Administrator:** The site shall select an "Administrator" whom will be the person responsible for all high-level administration of the program. This person will control access to the program by adding new users, structuring the individual users authority levels, and reporting to management, as appropriate, on any unauthorized use of the program.
- **Personnel Qualifications:** Personnel that develop, maintain, or use electronic record/electronic signatures shall have the education, training and experience necessary to perform their assigned tasks.
- **Written Policies:** There shall be written policies which hold individuals accountable and responsible for action initiated under their electronic signatures, in order to deter record and signature falsification. There shall be written revision and change control procedures to ensure that the program is administered in compliance with the FDA's requirements.
- **Record Archiving and Deletion:** The site is responsible to ensure that archiving or other file management techniques are suitable such that electronic records generated by the system shall be accurate and readily retrievable. Records may be removed by deleting the entire shared database or individual files within the database. This action may be conducted by the Administrator or by any Power User.
- **User Identity:** Any person that will be authorized to use an electronic signature will have their identity confirmed by the Administrator prior to granting them program access. Only the genuine owner of the electronic signature is allowed to access the program through their ID/password combination. System users should be informed that accessing the program using someone else's logon is a violation of the FDA rule.

- **Password Expiration/Recall:** All passwords must be checked, recalled, or revised at an interval appropriate with the security needs of the organization. Personnel whom no longer work at the establishment shall have their program access capabilities deactivated in a timely manner.
- **Certification to FDA:** The site must certify to FDA that the electronic signatures utilized on records are intended to be as legally binding as handwritten signatures, prior to or at the time of record submission to FDA. The FDA rule should be consulted for details and method to be used.
- **Notification of Attempted Security Breaches:** The software utilizes an error log system to notify the Administrator of log-in failure incidents that exceed the limits they have established. The administrator is responsible to "immediately and urgently" notify the appropriate personnel at the site if the activity appears to be an attempt to breach security.
- **Signature Representations:** The administrator should be made aware that while electronic signatures representations cannot be excised or added they can be copied by screen copy techniques and pasted into other documents. These modified documents cannot be resaved in the secure program but may be printed out as is.

Verifying Minimum System Requirements

☑ **KC4 v3.4**

☑ **KC4 *Signature***

Before installing KC4, verify that the computer(s) meet or exceed the following requirements:

- Pentium processor / 100 MHz minimum *
- Microsoft Windows 95/98/NT/2000/XP operating system *
- 64 MB of RAM minimum *
- 30 MB of available hard drive space for the KC4 program files
- Additional available hard drive space for storing protocol and plate data files
- CD ROM drive
- Super VGA color monitor
- Keyboard and mouse ports
- Serial port (for autoreader connection)
- USB port (with USB-to-serial adapter, if required)

* For users with Microsoft® Word/Excel 2000 (or greater) and Windows 98/NT/2000/XP, **PowerReports** links KC4 with Excel and Word, providing a powerful format for creating custom publication-quality report and export documents. For the PowerReports feature to run successfully, the computer(s) must meet or exceed the following requirements:

- Pentium II processor / 300 MHz minimum
- Microsoft Windows 98/NT/2000/XP operating system
- 128 MB RAM recommended
- Microsoft Office 2000 (9.0.3821 SR-1 or newer), Office XP, Office 2003

Upgrading from an earlier version of KC4

☑ **KC4 v3.4**

☑ **KC4 *Signature***

Upgrade Notes:

- If you are upgrading from KC4 version 3.1, 3.2, or 3.3 and you plan to **uninstall** your existing version, we recommend you do so *after* successfully installing version 3.4. This will allow the 3.4 installation program to recognize your current registration information.
- You are not required to uninstall an earlier KC4 version. If you choose to uninstall a **pre-3.1** version of KC4, however, be sure to preserve the **KC4_32.pld** file found in the root KC4 folder. This file *may* contain custom plate formats that must be imported into the new plate database to support any protocols that reference custom plate formats. See "Plate Format" in *System Menu* to learn more.
- If you are upgrading from KC4 version 3.1, 3.2, or 3.3 and you plan to reuse existing Local, Shared, and/or Diagnostic databases, make a note of the names and current locations of these databases. They will have to be upgraded for use with version 3.4. During the version 3.4 installation, KC4 will automatically run a **Database Upgrade Utility** to do this. KC4 will search all fixed, non-removable drives (e.g., diskette, ZIP, JAZ, and network drives are *not* automatically searched), for the following files: KCLocal.mdb, KC*.mdb, and Calib.mdb. You will have an opportunity to specify additional databases for upgrade during the installation. You can also run the utility after KC4 is installed via the System|Setup, Database menu option.

  Note: When KC4 prepares to upgrade a database, it makes and saves a *copy* of the original. The original database will remain unchanged and it can still be used with the earlier KC4 version. The upgraded databases are not backwards-compatible. That is, a KC4 version 3.4 database will not be recognized by KC4 version 3.1.

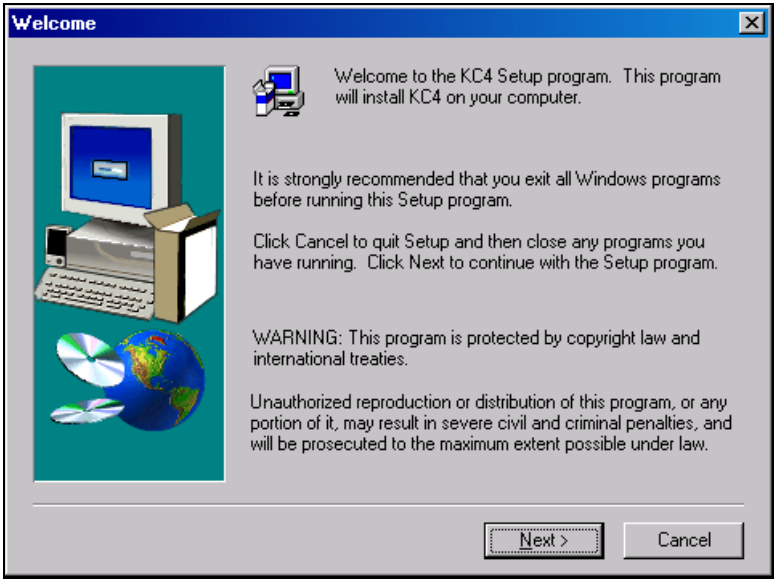
Installing KC4

☑ **KC4 v3.4**

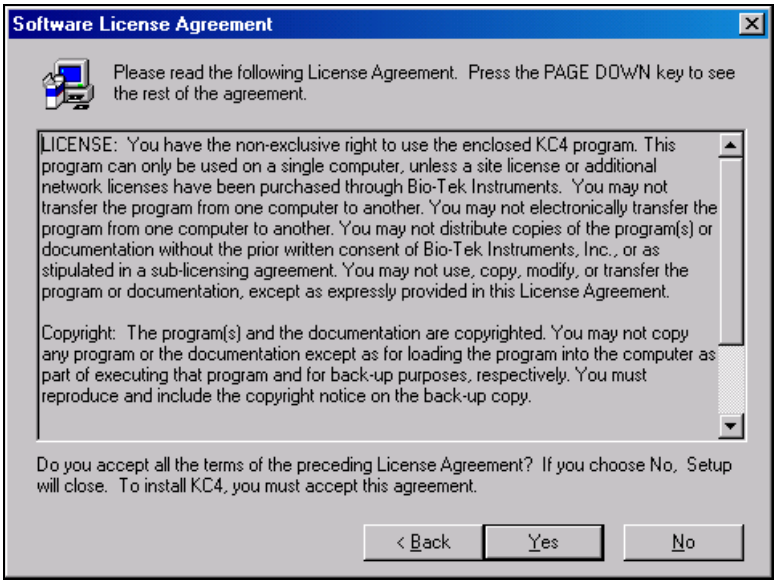
☑ **KC4 *Signature***

Follow these steps to install KC4:

1. Start Windows.
   - **Note:** If you are prompted with a Windows **password entry** dialog, enter a valid password; this helps to ensure a complete installation. If you were *not* prompted with the Windows password entry, disregard this note.
2. Close any unnecessary Windows programs.
3. Place the **CD** in the CD ROM drive.
4. Click the Windows **Start** button and select **Run.**
5. When the **Run** dialog appears, type `<drive>:\Setup.exe`, where `<drive>` represents the CD rom drive.
   - Alternatively, use the **Browse** function to access the drive and then click on `setup.exe`.
6. Click **OK.** After a brief pause, the **Welcome** dialog appears.
7. Click **Next** to continue. An informational dialog appears:

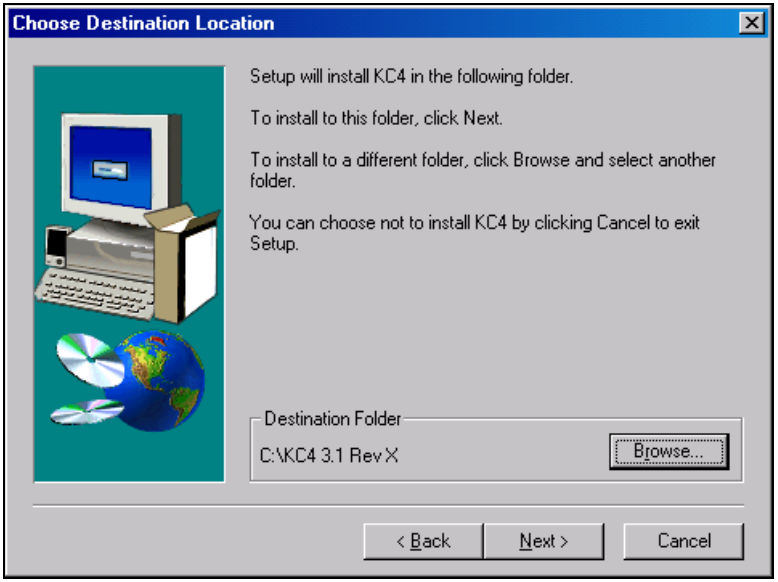

Welcome
Welcome to the KC4 Setup program. This program will install KC4 on your computer.
It is strongly recommended that you exit all Windows programs before running this Setup program.
Click Cancel to quit Setup and then close any programs you have running. Click Next to continue with the Setup program.
WARNING: This program is protected by copyright law and international treaties.
Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extent possible under law.
Next >
Cancel

8. Click **Next** to continue. The **Software License Agreement** dialog appears:


Software License Agreement
Please read the following License Agreement. Press the PAGE DOWN key to see the rest of the agreement.
LICENSE: You have the non-exclusive right to use the enclosed KC4 program. This program can only be used on a single computer, unless a site license or additional network licenses have been purchased through Bio-Tek Instruments. You may not transfer the program from one computer to another. You may not electronically transfer the program from one computer to another. You may not distribute copies of the program(s) or documentation without the prior written consent of Bio-Tek Instruments, Inc., or as stipulated in a sub-licensing agreement. You may not use, copy, modify, or transfer the program or documentation, except as expressly provided in this License Agreement.
Copyright: The program(s) and the documentation are copyrighted. You may not copy any program or the documentation except as for loading the program into the computer as part of executing that program and for back-up purposes, respectively. You must reproduce and include the copyright notice on the back-up copy.
Do you accept all the terms of the preceding License Agreement? If you choose No, Setup will close. To install KC4, you must accept this agreement.
< Back
Yes
No

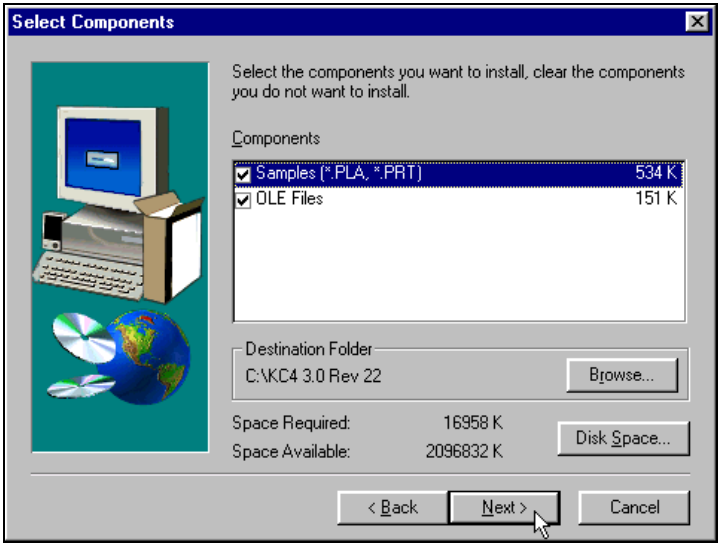
9. If you accept these terms, click **Yes** to continue. Otherwise, click **No** to discontinue the software installation.

   If you click **Yes,** the **Choose Destination Location** dialog appears:


Choose Destination Location
Setup will install KC4 in the following folder.
To install to this folder, click Next.
To install to a different folder, click Browse and select another folder.
You can choose not to install KC4 by clicking Cancel to exit Setup.
Destination Folder
C:\KC4 3.1 Rev X
Browse...
< Back
Next >
Cancel

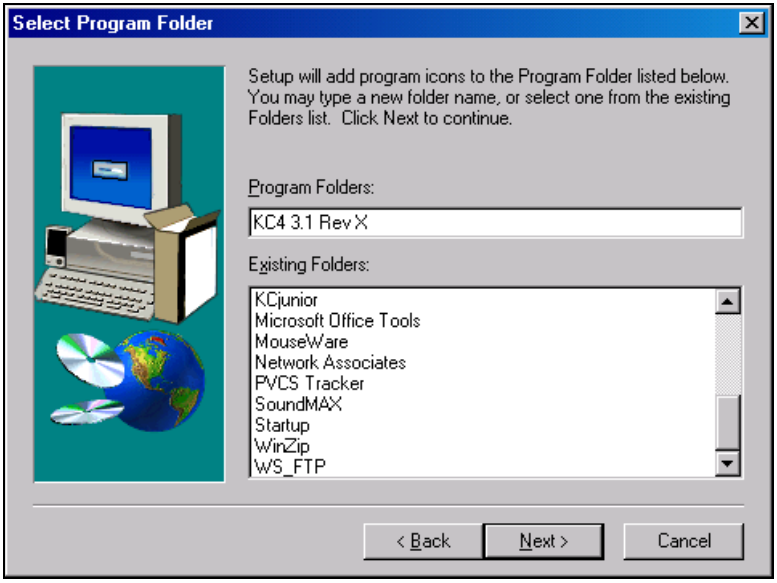
10. The default location for the software is displayed. Click **Next** to accept the default location, or click **Browse** to specify a different location.

    After a location is specified, the **Select Components** dialog appears:



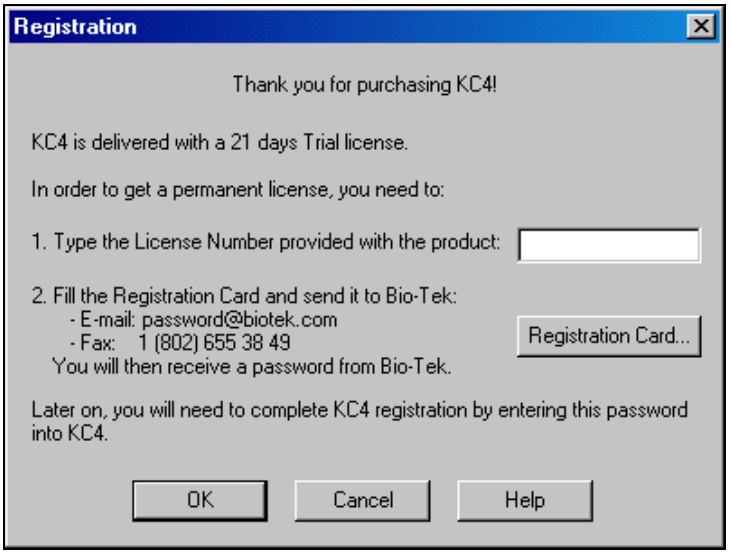
11. Check the components you want to install and click **Next.**

    The **Select Program Folder** dialog appears:

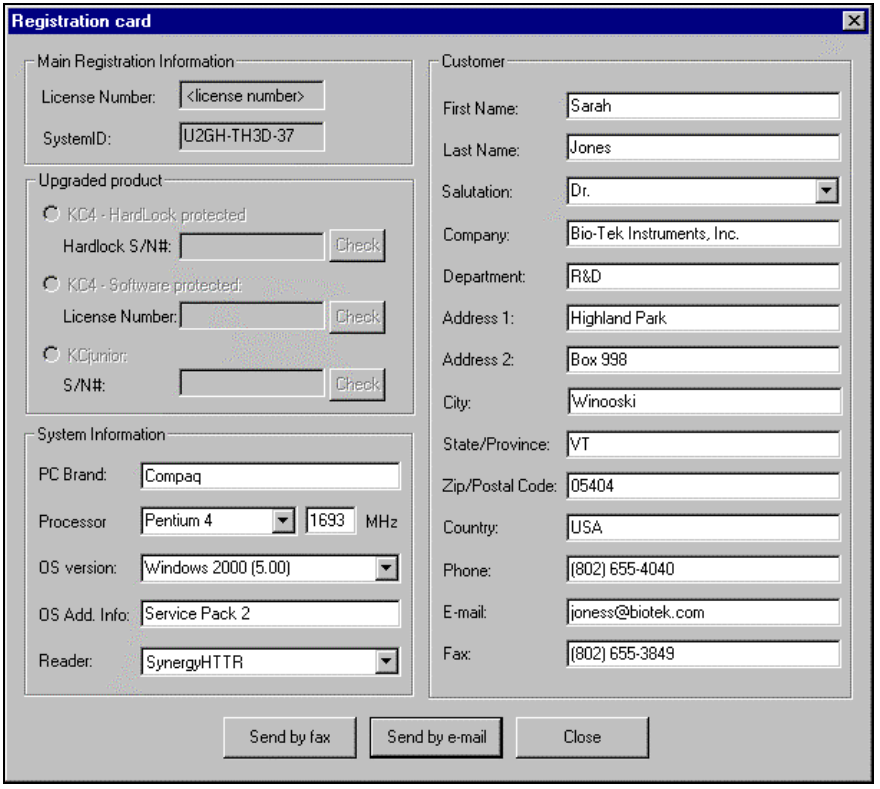


12. Accept the default **Program Folder** or enter a new one. Click **Next** to continue with the installation process.

After a few moments, the **Registration** dialog appears:


Registration
Thank you for purchasing KC4!
KC4 is delivered with a 21 days Trial license.
In order to get a permanent license, you need to:
1. Type the License Number provided with the product:
2. Fill the Registration Card and send it to Bio-Tek:
- E-mail: password@biotek.com
- Fax: 1 (802) 655 38 49
You will then receive a password from Bio-Tek.
Registration Card...
Later on, you will need to complete KC4 registration by entering this password into KC4.
OK
Cancel
Help

13. Enter the **License Number** provided with your KC4 package.
14. Click the **Registration Card** button to verify the number and open a registration form:



15. Enter the required information and click **Send by Fax** to print a copy of this form for later faxing, or **Send by E-mail** to send the information to Bio-Tek via email.
    - Bio-Tek will respond within two business days with your password.
    - If you are **upgrading** from a previous KC4 version or from KCjunior, enter the requested KC4 hardlock serial number, KC4 software license number, or KCjunior serial number, as appropriate. Click **Check** to verify the number.
    - If you are **reinstalling** KC4, you must submit a separate Reinstallation Form to obtain another password. Reinstallation is defined as (1) re-installing a KC4 version on the same PC (for example, after a hard disk replacement), or (2) un-installing a KC4 version from one PC and installing the same version on another PC.

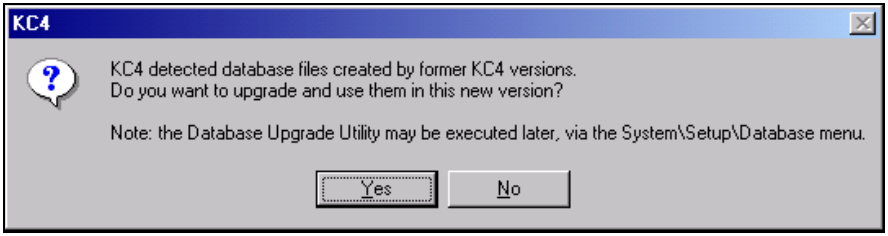
      A Reinstallation Form (.doc and .txt formats) complete with instructions can be found in the root KC4 folder when the installation process is complete.
16. Click **Close** to return to the Registration window and then click **OK** to continue.

    KC4 will automatically run the **Database Upgrade Utility.** This utility is used to update Shared, Local, and Calibration databases created with earlier versions of KC4 so that they will be compatible with the current version of KC4. For example, if you are upgrading from KC4 version 3.1 to 3.4, your version 3.1 databases must be converted before they can be used by KC4 version 3.4.

Database Upgrade Utility
KC4 is searching for databases used by former versions...
Scanning:
C:\Documents and Settings\All Users\Application Data\Bio-Tek Instruments
Stop

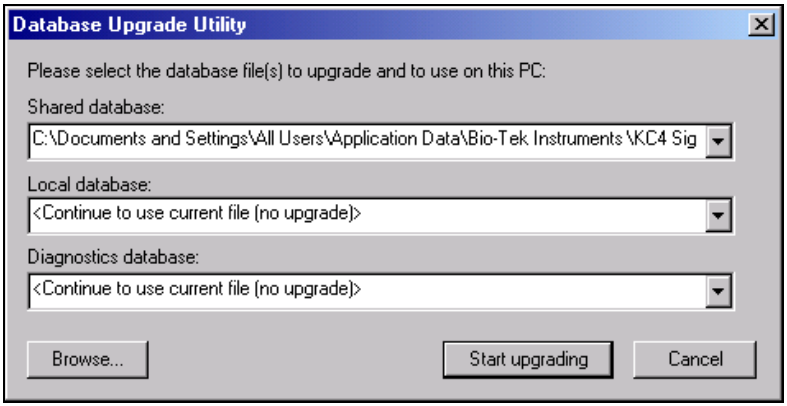
    - KC4 automatically begins a search of the fixed, non-removable drives (e.g., diskette, ZIP, JAZ, and network drives are not automatically searched). KC4 searches for the following files: KCLocal.mdb, KC*.mdb, and Calib.mdb. **Note:** This automatic search will not find Shared database files if their names do not begin with "KC".
17. If you know the names and locations of the databases you wish to upgrade, and/or if the databases reside on a drive that is not automatically searched, click the **Stop** button to advance to the Database Upgrade Utility dialog (see next step).

    When the search is complete, if KC4 has detected database files created by former KC4 versions, an upgrade option message box appears:


KC4
KC4 detected database files created by former KC4 versions.
Do you want to upgrade and use them in this new version?
Note: the Database Upgrade Utility may be executed later, via the System\Setup\Database menu.
Yes
No

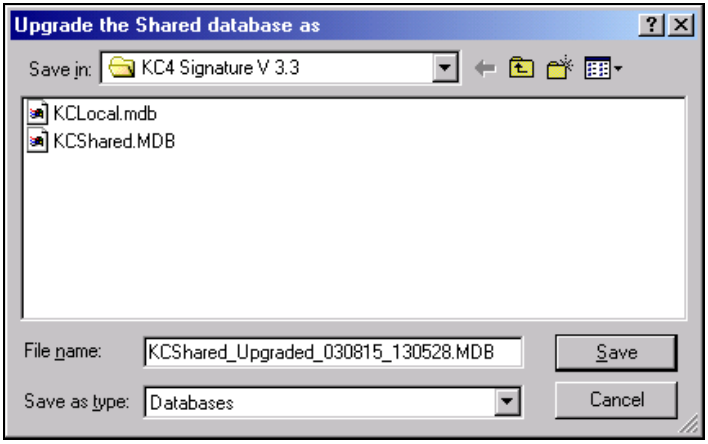
18. Click **Yes** to upgrade the database(s) now, or **No** to skip the upgrade at this time. The Database Upgrade Utility can be run manually through KC4, via System|Setup, Database.

If you click Yes, the **Database Upgrade Utility** dialog appears:



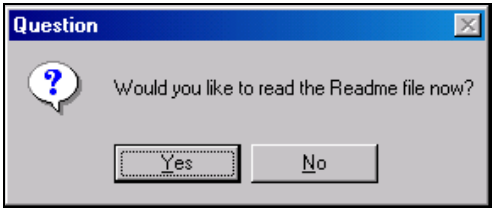
- Click the drop-down arrows to see which databases KC4 found. Click on the desired database name to select it for upgrading.
- Click the **Browse** button to search for a database. When the database is located, highlight it and click Open. KC4 will insert the name in the appropriate field (Shared, Local, or Diagnostics).

19. Click **Start Upgrading** to begin the process. When prompted, enter name(s) for the converted databases. (The original databases will remain unchanged). Default names contains a date and time stamp, as shown below.



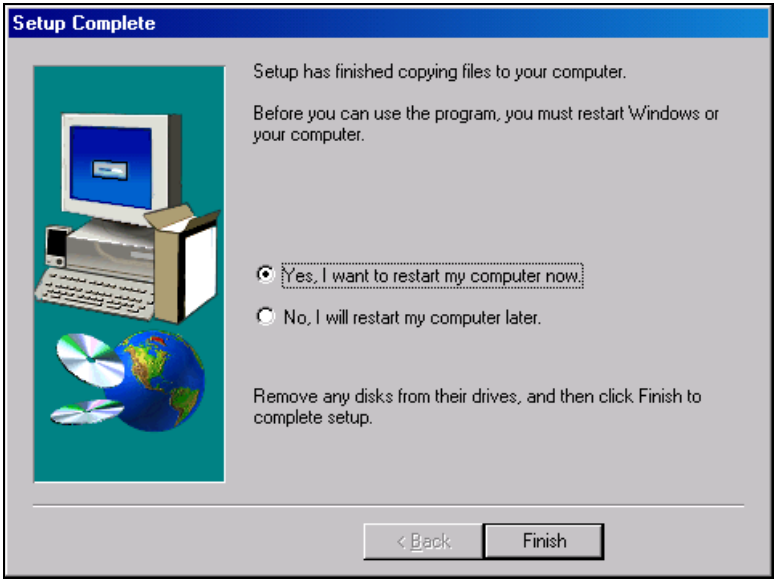
20. Click **Save** to complete the upgrade.

When the process is complete, KC4 will ask if you would like to read the Readme file:



21. Click **Yes** or **No** as desired.

    If you click No, or when you exit the text editor, the installation process finishes and then the **Setup Complete** dialog appears:


Setup Complete
Setup has finished copying files to your computer.
Before you can use the program, you must restart Windows or your computer.
Yes, I want to restart my computer now.
No, I will restart my computer later.
Remove any disks from their drives, and then click Finish to complete setup.
< Back
Finish

22. Remove the CD from the drive and click **Finish** to restart the computer.

Starting KC4

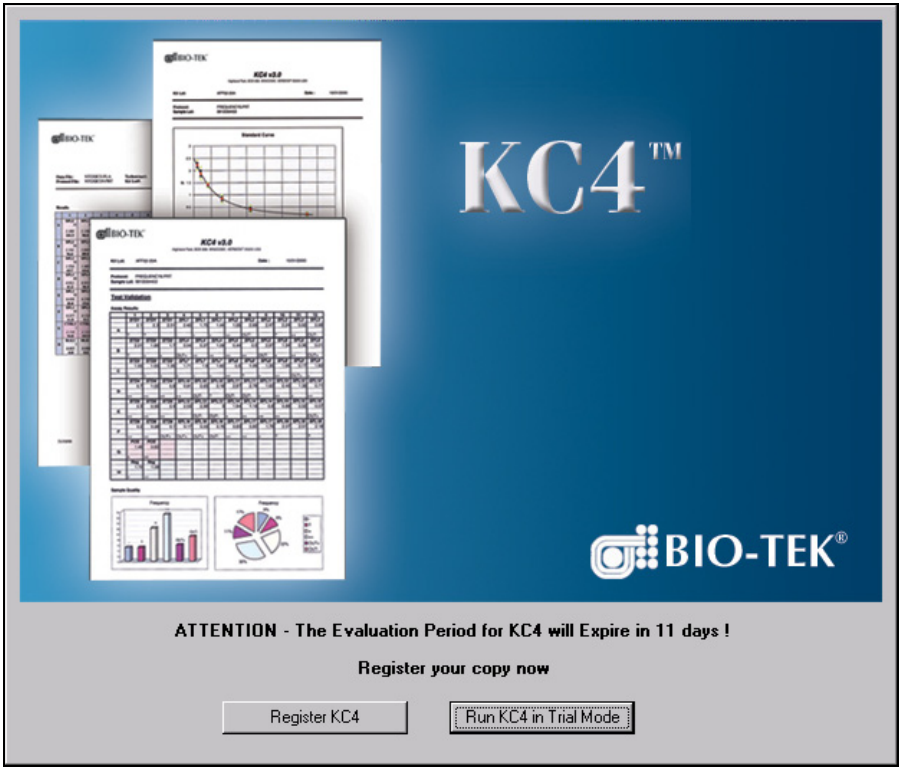
☑ **KC4 v3.4**

☑ **KC4 *Signature***

To start KC4:

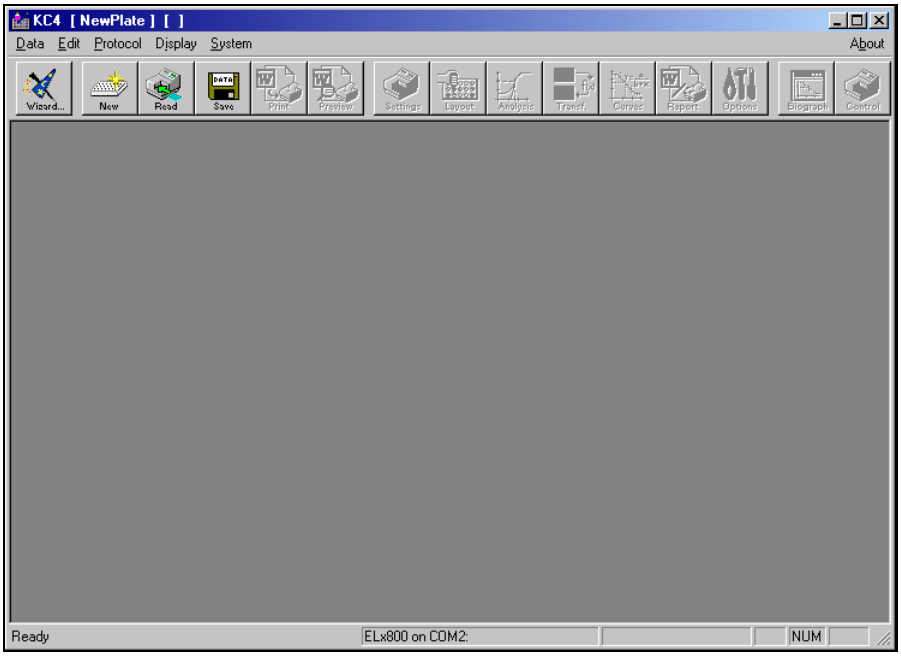
1. Double-click on the KC4 icon on the desktop, or open the **Start** menu and select **Programs** > **KC4 3.4 Rev #** > **KC4**.

   KC4's opening dialog appears (KC4 *Signature* users will see a different picture):


KC4™
BIO-TEK®
ATTENTION - The Evaluation Period for KC4 will Expire in 11 days !
Register your copy now
Register KC4
Run KC4 in Trial Mode

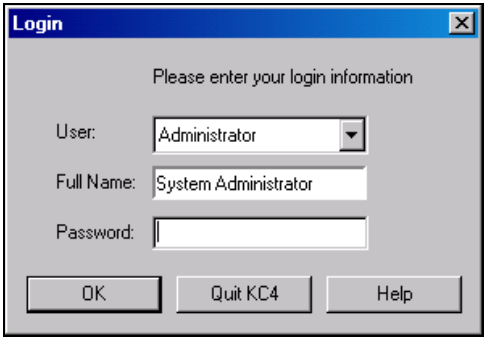
2. Click **Run KC4 in Trial Mode.**

KC4's main dialog appears:



Note: KC4 may attempt to communicate with an attached microplate reader. If you have not yet attached a reader, do not be concerned at this time if messages resembling "NO READER RESPONSE" and "Error when getting wavelength table from reader" appear. These messages will disappear when a reader is properly connected and configured.

KC4 *Signature* users will be presented with a Login dialog:



- Enter **"admin"** in the Password field and click **OK.** The dialog will disappear and "System Administrator" will appear in KC4's status bar at the bottom of the dialog.

TplRep_1 Error Message

Under certain circumstances, an error message resembling 'TplRep_1.xla could not be found' may appear in KC4. It is most likely to appear if an earlier version of KC4 was uninstalled and the remaining KC4 root folder was moved, renamed, or deleted.

This error message occurs because one of the files used for building PowerReports cannot be found. The error may occur:

- When starting KC4, if Excel is the default reporting or exporting engine and the Preferred file format is GLB.
- When creating a new protocol or GLB file, if Excel is the default reporting or exporting engine.

Follow these steps to solve the problem:

1. Close KC4.
2. Launch Microsoft Excel. The 'TplRep_1.xla could not be found' message will appear.
3. Click OK.
4. In Excel, select Tools/Add-Ins. The 'TplRep_1.xla' Add-In will be selected.
5. Unselect the 'TplRep_1.xla' Add-In. Excel will display an error message indicating that the file could not be found and prompts you to delete this Add-In.
6. Click Yes to delete the Add-In.
7. Quit Excel.
8. Restart KC4. The 'TplRep_1.xla could not be found' message should no longer appear.

Changing the System Administrator's Password

☑ **KC4 v3.4**

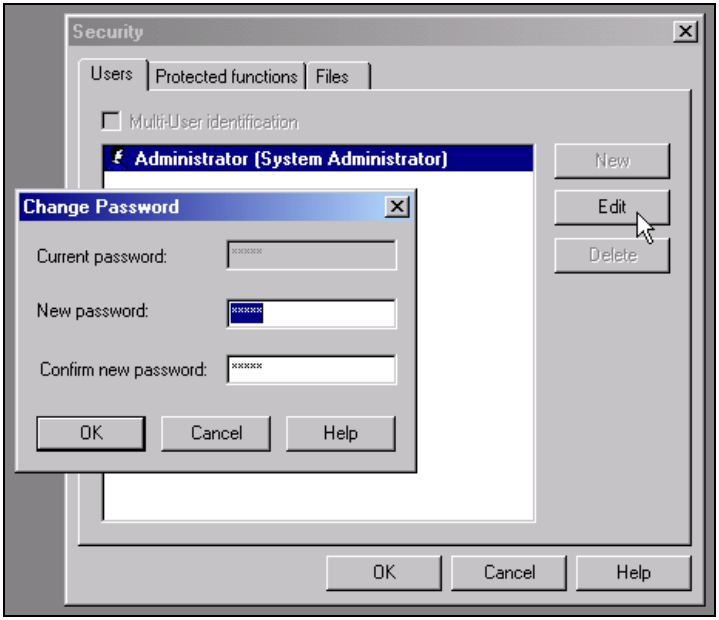
☑ **KC4 *Signature***

We recommend that you change the System Administrator's password immediately following software installation to ensure a secure operating environment.

To change the System Administrator's password:

1. Log in as the System Administrator if you have not already done so.
   - KC4 v3.4: Select System|**Administrator LogIn.**
   - KC4 *Signature*: Select System|**LogIn/LogOut.**
2. When the **Login** dialog appears, enter the password **"admin"** and click **OK.**

3. Select System|**Security** and click the **Edit** button.



4. Enter a **new** password, **confirm** the password, and then click **OK.**
   - **Important!** Do not forget the Administrator's password. If you do, you will have to reinstall KC4.

Copying KCShared.mdb to a Secure Network Location

☑ **KC4 v3.4 (optional)**

☑ **KC4 *Signature***

KCShared.mdb is a database file installed by KC4.

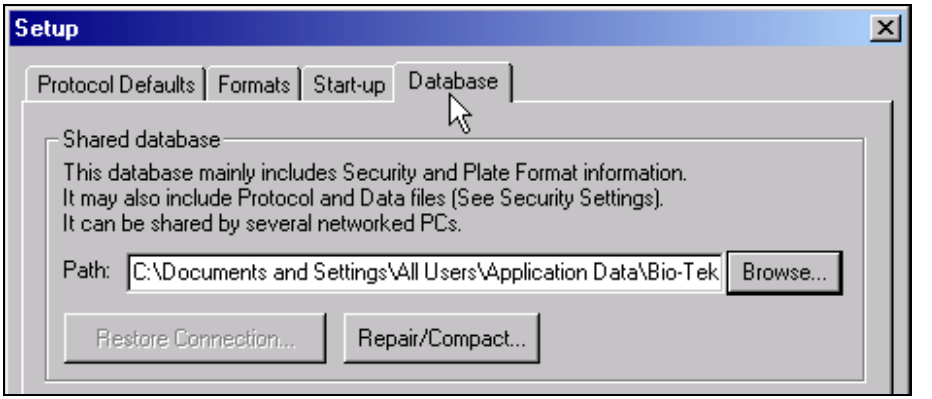
- With KC4 v3.4, KCShared contains security and plate format information.
- With KC4 *Signature*, KCShared contains security and plate format information; user accounts; protocol, plate data, and global data files and their associated audit trails; and a system audit trail for shared events.

Following installation, KCShared.mdb is stored on the computer's hard drive but a copy of it can be stored in a network location for secure record storage and for multi-user access.

Note: It is important to keep either the original KCShared.mdb file or a copy of the network file on each user's hard drive in case the user needs to revert to it following an extended network connection failure.

To locate the installed KCShared.mdb file and copy it to the network:

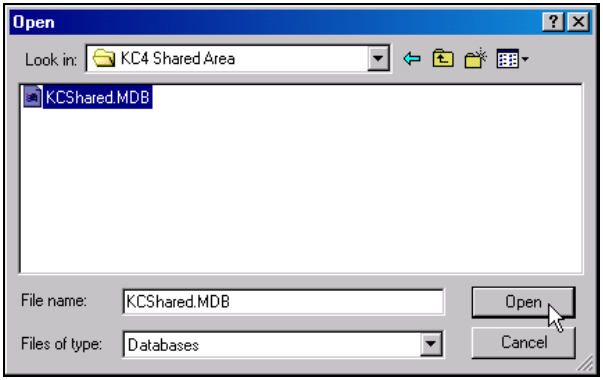
1. In KC4, select **System|Setup** and click the **Database** tab.



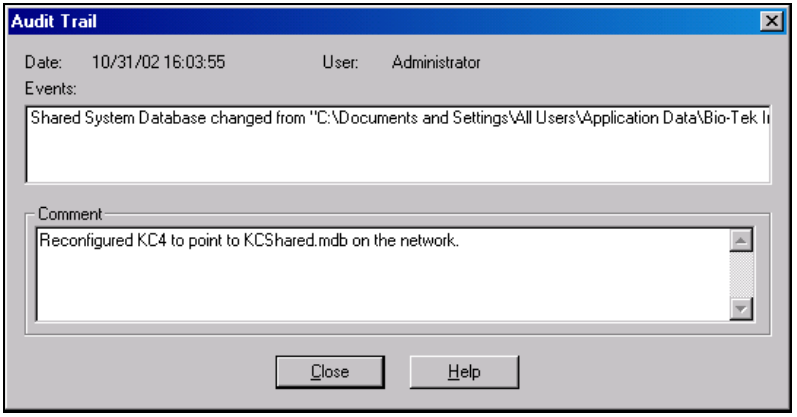
2. The **Path** field in the Shared Database section contains the current location of KCShared. (To see the full pathname, click once in the Path field and press the right arrow key.)
3. Launch Microsoft Explorer and go to this location.
   - **Tip:** With some operating systems, KCShared.mdb is installed in the **Application Data** folder under \Documents and Settings\All Users. If you are looking for the Application Data folder on your computer and can't find it, it is probably hidden. To reveal this folder, open Microsoft Explorer and select Tools|Folder Options|View. Make sure that 'Show hidden files and folders' is enabled.
4. **Copy** KCShared.mdb to the clipboard and **paste** it in a secure, shared-access location on the network.
5. Return to KC4.

To configure KC4 to use the KCShared.mdb file on the network:

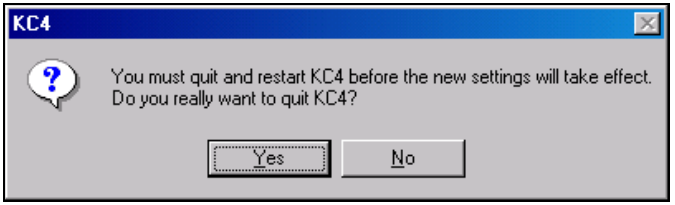
1. Within the System|Setup, Database dialog, click the **Browse** button. A standard Windows-style **Open** dialog will appear.



2. Use the file tree to locate the KCShared.mdb file on the network. Highlight the file and click **Open.** The Database dialog will reappear and the **Path** field will update to show the new location.
3. Click **OK** to close the Database dialog.
   - **Note:** With KC4 *Signature*, an **Audit Trail** dialog will appear to log this event. Enter an appropriate comment and click **Close.**



4. KC4 will display the following message. Click **Yes** to quit KC4.



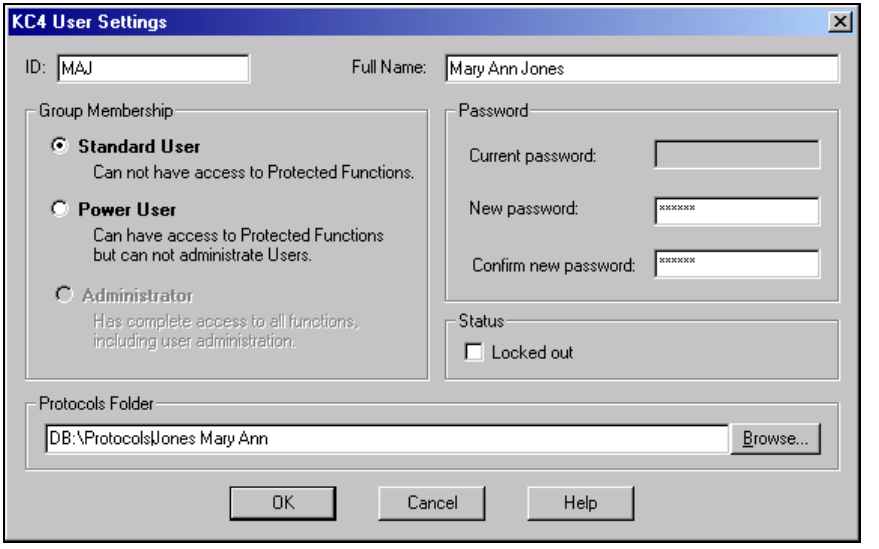
5. Restart KC4.

Creating New User Accounts

☑ **KC4 *Signature***

KC4 *Signature* supports the creation of individual **user accounts** to ensure that only authorized users can gain access to the system and to any protected functions. To create a new user account:

1. Select **System|Security|Users.**
2. Click **New.** The KC4 User Settings dialog will appear.



3. Enter a unique **ID** using 1 to 16 alphanumeric characters. The user will enter or select this ID when logging into KC4 and when signing files.
4. Enter the user's **Full Name.** This name will be associated with events logged by this user's actions and with signatures applied by this user.
5. Choose a **Group Membership.**
   - *Standard Users* are denied access to protected functions. (The System Administrator determines which functions shall be protected from use by Standard Users.)
   - *Power Users* are permitted access to protected functions. Power Users cannot administer user accounts or modify Security settings.
6. Enter a temporary **password** for the user in the **New** and **Confirm** fields.
   - Share the password with the user with instructions to change it as soon as possible.

7. (Optional) Specify a folder location from which the user will most often access protocol files. When the user selects Data|New Plate to initiate a plate read, the protocol selection dialog will default to this **Protocols Folder.**
   - The user is permitted to modify his or her Protocols Folder.
8. Click **OK** to save the changes and return to the Security dialog.

Connecting Reader(s) to the Computer

☑ **KC4 v3.4**

☑ **KC4 *Signature***

Before KC4 can be used to communicate with a microplate reader, the reader must be properly connected to the computer on which KC4 is installed.

The following briefly describes how to connect the reader to the computer. For more detailed information on reader setup, refer to the reader's user manual.

1. Attach the appropriate cable or adapter to an available serial or USB port on the back of the computer.
2. Plug the other end of the cable to the serial or USB port on the reader.
3. Connect the power cable to the back or side of the reader.
4. Plug all power cords into grounded electrical outlets. Use of a surge protector is strongly recommended.
5. Use KC4 to set and test the communication parameters, as described under "Reader Selection" in the *System Menu* chapter.

Where to go from here

☑ **KC4 v3.4**

☑ **KC4 *Signature***

KC4 is a powerful software program with many features and customizable options. Learn more now by following these recommendations:

- Go to the **Getting Started** chapter and:
  - Review essential information on file types, plate data acquisition, data reduction, and more.
  - Learn about KC4's menu structure, and how to navigate the Help system.
  - Get started with the Quick Start.
  - Examine sample protocol and plate data files, and read the corresponding assay descriptions.
- Go to the **Data Menu** chapter and read "Working with Data Files" to learn how to access, generate, modify, and save plate and global data files.
- Go to the **Protocol Menu** chapter and read "Working with Protocols" to learn how to access, create, modify, and save protocol files.
- Go to the **System Menu** chapter and read:
  - "Security" to understand all of the security options available to the System Administrator.
  - "Protected Functions" to learn how to protect certain KC4 functions from use by unauthorized users.
  - "Database" to learn about the different databases used by KC4.
  - "Exporting/Importing Plate Formats" to update the plate format database to support pre-version 3.1 protocols that reference custom plate formats.
  - "Importing Files" to transfer your pre-version 3.4 protocol and data files into the shared-access database (KC4 *Signature* users only).

Getting Started

Overview

Welcome to KC4! **Getting Started** introduces some important concepts for KC4 users, including:

- A description of KC4's key features, under **Essential Information.**
- A tour of KC4's **Main Screen.**
- An overview of each menu's contents, under **Quick Reference Guides.**
- Tips on **Navigating the Help System.**
- A typical sequence of events under **Quick Start.**
- A Quick Start just for **Clarity Microplate Luminometer** users.
- Instructions for locating and working with **Sample Files.**

Essential Information

KC4 allows you to define instructions for controlling a microplate reader, acquiring measurement data from that reader, for reducing and analyzing the retrieved data, and for printing, exporting, and saving the results.

The minimum instructions required for reader control and plate data acquisition are called **reading parameters.** Reading parameters varying according to reader model but generally include reading type (endpoint, kinetic, spectrum), reading method (absorbance, fluorescence, luminescence), filters/wavelengths, shaking and incubation instructions, well monitoring instructions, plate geometry, and various pre-reading options including pathlength correction.

Reading parameters can be combined with data reduction instructions in a **protocol.** In addition to storing the reading parameters, a protocol can store plate layout information, single plate transformations, multi plate transformations, curve-fitting parameters, cut-off and validation criteria, report and export templates, security settings, and more.

The KC4 software package also contains the **Biograph** module for viewing and manipulating curves. Biograph can be used either alone or in conjunction with KC4.

File Types

KC4 uses several proprietary file formats to store information:

- **Protocol (*.PRT)** files contain instructions for controlling the attached microplate reader. Protocols also contain information required by KC4 to analyze plate measurement data.
- **Plate data (*.PLA)** files contain raw measurement values, plate description information (prompts, comments, date and time of reading), and sample names.
- **Global data (*.GLB)** files contain plate data *and* protocol information. The GLB file format is offered as an alternative to maintaining two separate protocol and plate data files, and is the required data file format for electronic signature support.

In addition, **Clarity™ Microplate Luminometer** users will work with Clarity protocol files, which use a **.BPF** extension. These files are referenced by KC4's PRT and GLB files and they contain the reading parameters required to control the luminometer.

File Storage

File storage options vary depending on site requirements and the current KC4 configuration.

- Files may be stored individually on the computer's hard drive, on a CD or diskette, or on a network to which the computer is attached. This is the storage method that users of KC4 version 3.0x and earlier are familiar with. Windows Explorer or a similar application can be used to view the file names and their locations, and to move, copy, rename, and delete files.
- With KC4 *Signature*, PRT, PLA, and GLB files may also be stored inside a secure, shared-access database. This database, named KCShared.mdb, can be stored on a user's computer or on a shared-access computer. KC4 provides a special file maintenance utility for viewing the file names and their locations, and for moving, copying, renaming, deleting, importing, and exporting files.

Record Retention and Archival

With KC4, users create and save protocol, plate data, and global data files containing valuable assay information and test results. KC4 does **not** automatically delete or archive any of these records. It is therefore the site's responsibility to establish a procedure and schedule for record retention and archival.

Important: Regardless of license type, KC4 stores information using three different databases: KCShared.mdb, KCLocal.mdb, and CALIB.mdb. See "Database" in *System Menu* to learn more about their functions and locations. Because file or network problems can potentially create unrecoverable database errors, these databases should be backed up on a regular basis.

- **KC4 v3.4 Users:**

  Protocol, plate data, and global data files are stored individually on a user's computer or on a network computer to which the user has access. Windows Explorer or a similar application can be used to backup/archive files by copying them to the desired medium.

- **KC4 *Signature* Users:**

  Protocol (PRT), plate data, and global data files are typically stored inside KCShared.mdb (they can also be stored outside this database, see "File Storage and Format Options" in *System Menu* for more information). To backup/archive files you can either copy the entire database to the desired medium, or you can export files to a disk and then copy them to the desired medium.

  - See "Maintain Files" in *System Menu* to learn more about exporting and optionally deleting files from the shared database.
  - See also "Audit Trail" in *System Menu* to learn about exporting and optionally deleting system audit trail events to reduce the size of KCShared.mdb.

Plate Data Acquisition

KC4 provides three methods for acquiring plate data: retrieving results from a microplate reader, entering plate data manually, and reading data in from an existing text file. Refer to the following for more information:

- "Reading Plates" on page 48
- "Entering Plate Data Manually" on page 50
- "Reading Plate Data from a File" on page 51

Data Reduction and Analysis

When the data has been retrieved and data reduction is complete, KC4 displays the raw data associated with the first defined filter/wavelength. The availability of additional **data sets** depends on the current protocol parameters and can include raw data at other filters/wavelengths, blanked data, corrected data, concentrations, symbols, kinetic curves, and more. Refer to the following for more information:

- "Results Overview" on page 251
- "Data Sets" on page 253

Biograph

The KC4 software package includes the Biograph module for curve viewing and manipulation. Biograph can be used either as a stand-alone product or in conjunction with KC4. See "Biograph" for more information.

File Signatures

KC4 *Signature* users can electronically **sign** protocol files and global data files. Signing a file is easy; select Protocol|Sign or Data|Sign and KC4 will guide you through the process. Electronic signatures are permanent and cannot be modified, and they remain a part of the overall protocol or global data file for the life of that file. Signatures can be viewed by selecting Data|Audit Trail or Protocol|Audit Trail.

Audit Trails

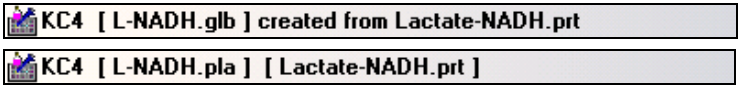
KC4 automatically logs events for user actions and stores these events inside audit trails:

- KC4 v3.4 maintains a Data Audit Trail for each plate and global data file. These audit trails contain events for Change and Mask actions applied to the measurement data.
- KC4 *Signature* maintains three types of audit trails: Protocol, Data, and System. These audit trails contain events for many user activities including login/logout, protocol and global data file creation and modification, plate reading, and more.

Main Screen

KC4's functions are controlled through the main screen, which contains a **Title Bar, Menu Bar, Toolbar** and **Status Bar.**

Title Bar



The title bar shows the current open file(s). Its appearance depends on the current **Preferred Data File Format** setting (selected via System|Security|Files).

When the Preferred Data File Format is set to **.GLB:**

- The set of brackets **[ ]** embraces the Global Data File name (extension **GLB).**
- If a previously-saved protocol was used to generate the .GLB file, the title bar includes the text "created from <protocol name>.prt"

When the Preferred Data File Format is set to **.PLA:**

- The first set of brackets **[ ]** embraces the Plate Data File name (extension **PLA).**
- The second set of brackets **[ ]** embraces the Protocol File name (extension **PRT).**
- An **asterisk** (*) next to any file name indicates that its contents have changed but the file has not yet been saved.

Menu Bar

- The **Data** menu contains functions for acquiring and processing measurement data.
- The **Edit** menu provides tools for copying data from the screen in KC4 and pasting it into a spreadsheet, word processor, database manager, or text editor. (Data *cannot* be pasted back into KC4.)
- The **Protocol** menu contains functions for creating, modifying, and saving assay protocols.
- The **Display** menu provides access to the Biograph module and also displays the results of the Validation criteria defined in the protocol.
- The **System** menu contains special functions for configuring readers, maintaining user accounts, configuring security parameters, setting global parameters, performing reader diagnostics, and more.
- On the far right of the menu bar, the **About** menu provides access to the Help system, Bio-Tek's home page, and the About KC4 screen.

Toolbar

The **Toolbar** contains buttons that, when clicked, provide quick access to KC4 functions. Accessibility of a particular toolbar button depends on whether or not its function applies to the current environment. For example, the Biograph button is accessible only if results are displayed and a curve has been generated. When KC4 is installed, the toolbar contains a set of default buttons. These buttons can be customized by selecting **System|Toolbar.**

Status Bar

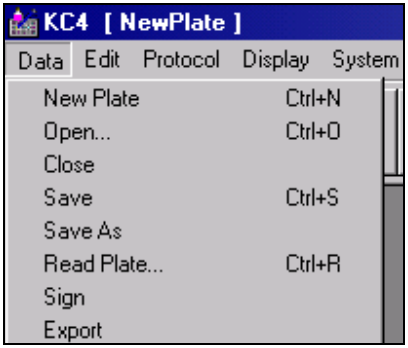
The **Status Bar** at the bottom of the main screen displays the current function, reader and COM port; the name of the person currently logged in (if applicable); and any enabled keyboard functions.

Quick Reference Guides

This section briefly describes the contents of each menu in KC4.

Data Menu

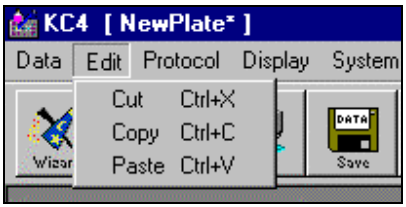
The **Data Menu** contains functions for acquiring and processing plate data.



New Plate	Clear memory to prepare to read a new plate.
Open...	Open an existing plate (.PLA) or global (.GLB) data file.
Close	Close the open data file. If the file has not yet been saved, KC4 provides the option to save before closing.
Save	Save the current plate data (.PLA) or global data (.GLB) file.
Save As	Save the open file under a new name.
Read Plate...	Read a plate using the attached reader, enter plate data manually, or read plate data in from a file.
Sign	Electronically sign the current .GLB data file.
Export	Export data to a text file, to an Excel spreadsheet, or to a Word document.
Append to Kinetic File...	Append the current set of plate data to an existing file.
Print...	Print a report according to the Protocol|Report settings.
Print Preview	Preview the report before printing.
Print Setup	Define the print setup before printing.
Plate Description...	Enter information about the current plate using up to six free-text fields plus a large comments field.
Samples...	Define the names of samples.
Audit Trail	View the contents of the Data Audit Trail.
Exit	Exit KC4.

Edit Menu

The **Edit Menu** contains editing functions commonly found in Windows.



Cut	Deletes selected data from the document and copies it to the Clipboard.
Copy	Copies selected data to the Clipboard.
Paste	Pastes data from the Clipboard into the document.

Protocol Menu

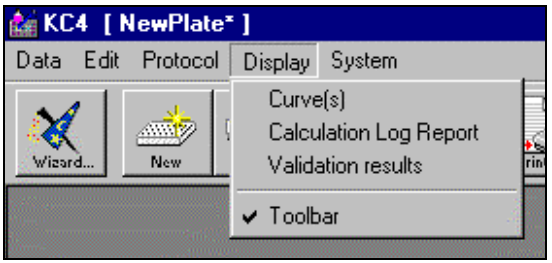
The **Protocol Menu** contains functions for creating, modifying, and saving assay protocols.

New	Create a new protocol.
Wizard	Launch the Wizard for help with creating protocols.
Open...	Open an existing protocol (.PRT) file.
Close	Close the current protocol file. If the file has not yet been saved, KC4 provides the option to save before closing.
Save	Save the current protocol (.PRT) file.
Save As...	Save the open file under a new name.
Sign	Electronically sign the protocol file.
Plate Description	Define the names of up to six free-text fields to appear at read time to allow the operator to enter relevant information, such as the site name and lot number.

Reading	Define the reading parameters, such as reading type, filters/wavelengths, shaking and incubation instructions, and plate type.
Raw Data Correction	Enable/Disable corrections to raw data. Options can include Blank Plate Subtraction, Blank Wells Subtraction, and Pathlength Correction.
Kinetic Analysis	Select the primary (and optional secondary) set of data for kinetic analysis, as well as the calculation type.
Spectrum Analysis	Select the data set for spectrum analysis, as well as the calculation type.
Scanning Analysis	Select the calculation type and related options for linear scanning analysis.
Plate Layout	Configure the layout, names, and colors of blanks, standards, controls, and samples. Define expected concentrations or dilutions.
Single Plate Transformations	Define and apply formulas to one or more wells within the plate.
Multi-Plate Transformation	Define calculations between different sets of plate data.
Curves	Define curve-fitting parameters.
Validation	Define the criteria used to assess the validity of the results or the suitability of calibrators for inclusion in subsequent calculations.
Cutoffs	Define the conditions used to screen selected data into defined categories based on fixed or variable threshold limits.
Report	Customize the contents and appearance of the report.
Export	Customize the contents and format of the data export file.
Audit Trail	View the contents of the Protocol Audit Trail.
Security	(Administrator and Power Users) Specify which protocol- and plate-specific functions, if any, shall be protected from use by Standard Users.
Options	Select automated functions. Choose the reporting and exporting engines. Enable the protocol audit trail function.
Advanced	Select Advanced to access special protocol menu options: Plate Description, Raw Data Correction, Multi Plate Transformations, Validation, and Cutoffs.

Display Menu

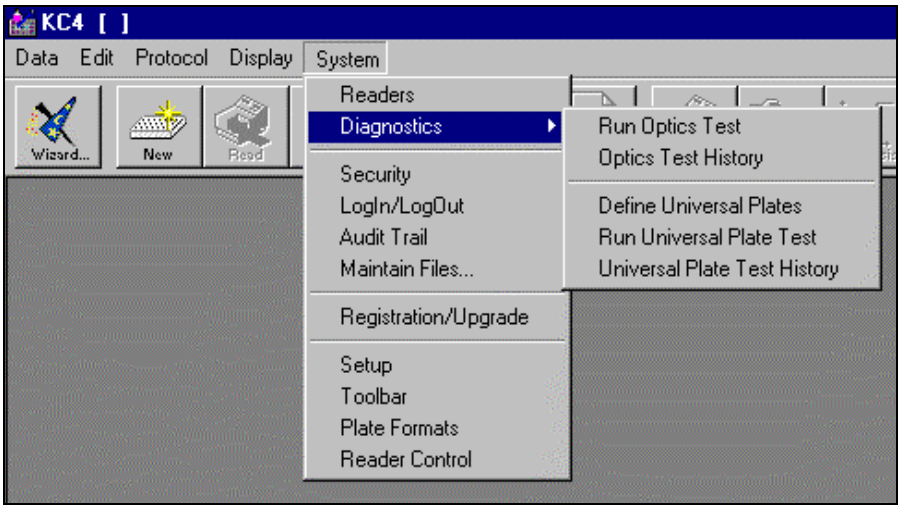
The **Display Menu** provides access to the Biograph module and also displays the results of the Validation criteria defined in the protocol.



Curve(s)	Launch Biograph and view the curve(s).
Calculation Log Report	View calculation warnings associated with the current data.
Validation Results	Check the status of every Validation condition defined in the protocol.
Toolbar	Show or hide the toolbar.

System Menu

The **System Menu** contains special functions for configuring readers, configuring system security, setting global parameters, performing reader diagnostics, and more.

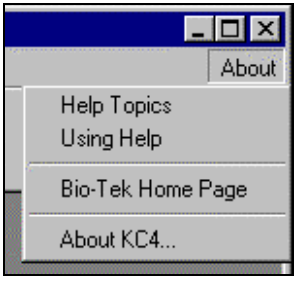


Readers	Change or configure the current reader.
Diagnostics	Run an Optics Test or Universal Plate Test on the attached reader. Define characteristics for a Universal Test Plate. View historical test results.

Security	Configure system security parameters and maintain user accounts.
Administrator LogIn/LogOut	Log into KC4 as the System Administrator (single-user system).
LogIn/LogOut	Log into KC4 using a unique ID and password combination (multi-user system).
Audit Trail	View the contents of the System Audit Trail.
Registration/ Upgrade	Enter a license number and password to ensure continuous use of KC4 after the trial period is over.
Setup	Set up global parameters including the import/export text separator, time format for kinetics, weighting of standard deviation, and Word/Excel options. Tell KC4 whether or not to use out-of-range values in statistics.
Toolbar	Customize the appearance of the toolbar by adding/removing icons.
Plate Formats	Create and modify plate formats, and specify a default format for new protocols.
Reader Control	Send a command to the reader to open or close the door, insert or eject the plate, and to control the temperature.

About Menu

The **About Menu** provides access to the Help system, Bio-Tek, and the About KC4 screen.

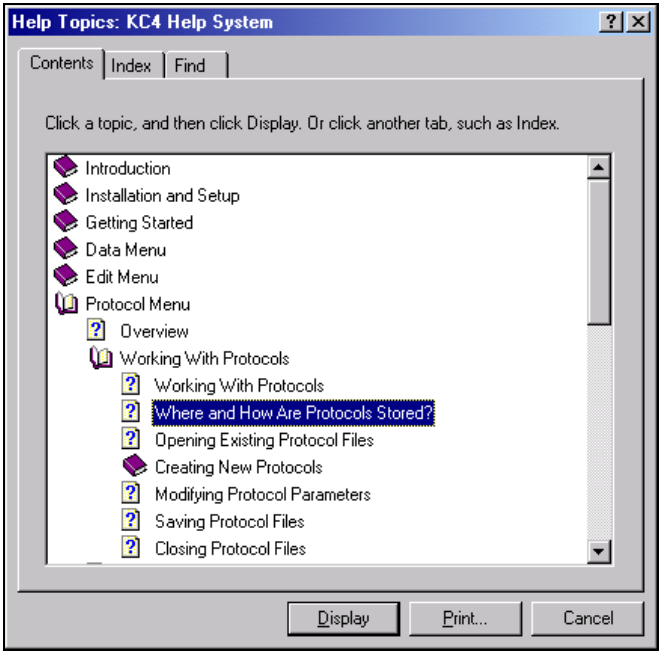


Help Topics	Open the Help window with its Contents, Index, and Find functions.
Using Help	Tips on using the Help system.
Bio-Tek Home Page	Automatically launch the browser and go to Bio-Tek's home page (www.biotek.com).
About KC4	Check the software version and revision numbers, view current system performance parameters, and find telephone and fax numbers for technical assistance.

Navigating the Help System

Most KC4 dialogs contain a **Help** button that, when clicked, opens an informational Help dialog. In addition to this **context-sensitive** help, KC4 provides access to the entire Help system.

Select **Help Index** from the **About** menu to open the Help Topics dialog.



- Double-click on a book to see a topic's sub-topics.
- Double-click on a page to view the text for a topic.
- Highlight a topic then click **Open** to view the text for the topic.
- Click the **Index** tab to perform a keyword search.
- Click the **Find** tab to search the entire help system for a specific word or phrase.
- Click **Print** to print the text for a topic.
- Click **Cancel** to close the Help Topics dialog and return to KC4.

Quick Start

When using KC4, you will typically follow some version of the steps shown below:

1. Connect a microplate reader to the computer (if not already connected).
2. Start KC4 and log in (if required).
3. Select **System|Readers** to define reader setup parameters and establish communication.
4. Select **System|Setup** to define global parameters, including special Word/Excel reporting options.
5. Select **Data|New Plate** to prepare to read a plate.
6. Open an existing protocol or begin with an empty protocol.
7. Select **Protocol|Reading** to define **reading parameters** such as wavelengths, plate type, and temperature.
8. Return to the main view and define the data reduction parameters:
   - create the **plate layout** by defining and assigning well identifiers
   - select **curve** parameters
   - specify **cut-off** and **validation** criteria
   - create single- and multi-plate **transformations**
   - define **reporting** and **exporting** options
9. Select **Data|Read Plate** to read a microplate.
10. Review the **results.**
11. Select **Data|Print** to print reports.
12. Select **Data|Save** to save the information, or with KC4 *Signature* select **Data|Sign** to electronically sign and then save the information.
13. Select **Display|Curves** to view the curve.

Quick Start for Clarity™ Microplate Luminometer Users

Clarity users will follow a slightly different sequence of events:

1. Connect the luminometer to the computer (if not already connected).
2. Start the Clarity software and set up the communications port.
3. **Important:** Close the Clarity software.
4. Start KC4 and log in (if required).
5. Select **System|Readers** and set **Clarity** as the current reader.
6. Select **System|Setup** to define global parameters, including special Word/Excel reporting options.
7. Select **Data|New Plate** to prepare to read a plate.
8. Open an existing **KC4 protocol** or begin with an empty one.
9. Select **Protocol|Reading** to open or create a **Clarity Protocol File** and define its reading parameters. (See "Parameter Input" in Chapter 4 of the Clarity Operator's Manual to learn about the reading parameters.)
10. Return to KC4's main view and define the data reduction parameters:
    - create the **plate layout** by defining and assigning well identifiers
    - select **curve** parameters
    - specify **cut-off** and **validation** criteria
    - create single- and multi-plate **transformations**
    - define **reporting** and **exporting** options
11. Select **Data|Read Plate** to read a microplate.
12. Review the **results.**
13. Select **Data|Print** to print reports.
14. Select **Data|Save** to save the information, or with KC4 *Signature* select **Data|Sign** to electronically sign and then save the information.
15. Select **Display|Curves** to view the curve.

Sample Files

KC4's installation program copies many sample protocol (PRT) and plate data files into a special SAMPLES folder inside the root KC4 folder and, with KC4 *Signature*, inside the KCShared database. Examine these files to learn how to create KC4 protocols to support your assays. The sample files cover a range of protocol options, including single plate transformations, multi plate transformations, curve fitting parameters, cutoff criteria, and more.

Important! The sample protocols must be considered as *examples* provided for demonstration and guidance purposes only. If you plan to use these protocols or similar ones in a real application, *it is your responsibility to validate the protocol parameters*, including the Excel sheet content (if PowerReports is used), before using them.

- The SAMPLES folder in the KC4 root folder also contains a **Sample Protocol Descriptions.doc** file describing the protocols.
- Files with the extension **.PRT** represent protocol files.
- Files with the extension **.PLA** represent plate data files.
- Files with the extension **.GLB** represent global data files (containing plate data and protocol parameters).
- Open protocol and plate data files with similar names, such as Quantitative.prt and Quantitative.pla. **Note:** Not every .PRT file has a corresponding .PLA file.

Data Menu

Overview

The **Data Menu** contains functions for acquiring, processing, and storing plate data.

This chapter covers the following topics:

- Creating, opening, saving, and closing data files
- Reading plates
- Entering plate data manually
- Retrieving plate data from an external file
- Applying electronic signatures to global data files
- Appending a new set of plate data to an existing kinetic file
- Exporting plate data
- Printing and previewing reports
- Entering descriptive information for the plate
- Entering sample names
- Viewing the data file's audit trail

Working with Data Files

This section describes accessing, generating, modifying, and saving files that contain plate measurement data.

Where and How is Plate Data Stored?

In KC4, there are two types of files used for storing plate data:

- **Plate data** files contain raw measurement values, plate description information (prompts, comments, date and time of reading), and sample names.
  - Plate data files are identified by the extension **.PLA** (DNA-Quant.pla)
- **Global data** files contain plate data *and* protocol information. The GLB file format is offered as an alternative to maintaining two separate protocol and plate files, and is the required data file format for electronic signature support.
  - Global data files are identified by the extension **.GLB** (DNA-Quant.glb)

File storage options vary depending on site requirements and the current KC4 configuration.

- Files may be stored individually on the computer's hard drive, on a CD or diskette, or on a network to which the computer is attached. This is the storage method that users of KC4 version 3.0x and earlier are familiar with. Windows Explorer or a similar application can be used to view the file names and their locations, and to move, copy, rename, and delete files.
- (KC4 *Signature*) Files may also be stored inside a secure, shared-access database. This database, named KCShared.mdb, can be stored on a user's computer or on a shared-access computer. KC4 provides a special file maintenance utility for viewing the file names and their locations, and for moving, copying, renaming, deleting, importing, and exporting files.

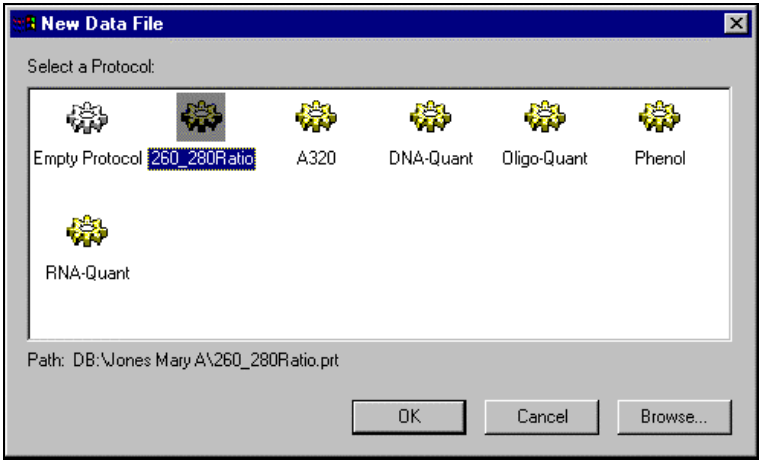
Creating a New Data File

Before a plate can be read, and before plate data can be entered manually or read in from a file, a **new data file** must be created.

To create a new data file:

1. Select **System|Readers** and set the **Current Reader** to the appropriate target reader model.
2. Select **Data|New Plate** (or click the toolbar icon shown above) to clear memory to prepare to read a new plate.
3. If KC4 is currently configured* to create plate data **(PLA)** files by default, do one of the following:
   - Select **Protocol|New** to create a new protocol.
   - Select **Protocol|Open** to open an existing protocol.
   - Select **Data|Read Plate** to initiate the plate read.

   If KC4 is currently configured* to create global data **(GLB)** files by default, selecting Data|New Plate opens the **New Data File** dialog:



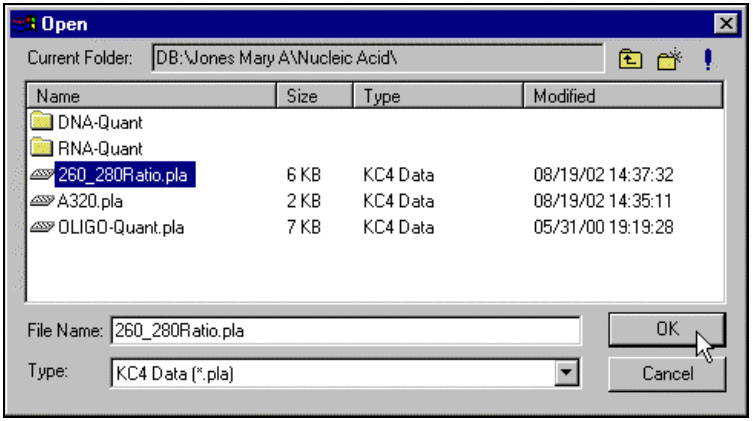
   This dialog allows you to choose which protocol to open and use for the plate reading.
   - Click the **Browse** button to search through the folder structure.
   - Highlight the desired **protocol** and click OK.
   - To start with an empty protocol and customize its parameters, highlight **Empty Protocol** and click OK.

* The "Preferred Data File Format" is selected via System|Security|Files.

Opening Existing Data Files

Select **Data|Open** to open a previously saved plate or global data file.

Note: The layout of the **Open** dialog varies slightly depending on whether or not the Secured Protocol and Data File Storage option is enabled, but the functionality is the same. (In the example below, this option is *enabled*.)



- The **Current Folder** field or the **Look In** field (you will see one or the other) shows your current location within the overall folder structure.
  - If the drive is represented as **DB:**, this means that files are currently being stored in KC4's secure, shared-access database. If the drive is represented by a single letter, such as **C:** or **D:**, this means that files are stored individually on the computer's hard drive or on a network to which the computer is attached. See "Where and How is Plate Data Stored?" for more information.
- Click to move up one folder level.
- Click on a folder to open it and view its contents.
- Click the **Type** drop-down arrow to change the type of files being viewed. Options include:
  - KC4 Data (*.PLA)
  - KC4 Data+Protocol (*.GLB)
  - All Files (*.*)
- Click on a file and click **OK** to open it.
- Click to update the view to show any folders/files that may have been added by another user after you opened this dialog.

Modifying Data Files

After data has been retrieved from the reader (or entered manually, or read in from a file), KC4 permits authorized users to change or mask raw measurement values. KC4 automatically logs an event to the file's Data Audit Trail any time a change or mask action occurs.

Note: Data Audit Trail behavior differs between KC4 v3.4 and KC4 *Signature*. Turn to page 63 to learn more.

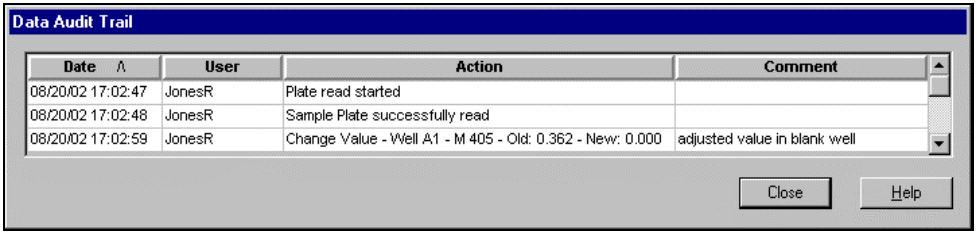
Important! Changing or masking wells may significantly alter or even invalidate results.

- Only raw measurement values can be changed. Raw data set names resemble M 405, M#3 490, and M 485/528.
- When a well is masked, KC4 excludes its raw measurement value from further calculations.

To **change** a raw measurement value within any data file:

1. Open the desired file.
2. Within the Results window, click the **Data** drop-down arrow and select a data set that contains raw measurement values (e.g. M 405 or M#2 485/528).
3. Click the **Change** box.
   - The System Administrator can **protect** the use of the Change function. If you are trying without success to use this function, you may not be authorized to use it.
4. Click on a well in the grid and change its value.
5. When the changes have been made, click **OK** to recalculate results.

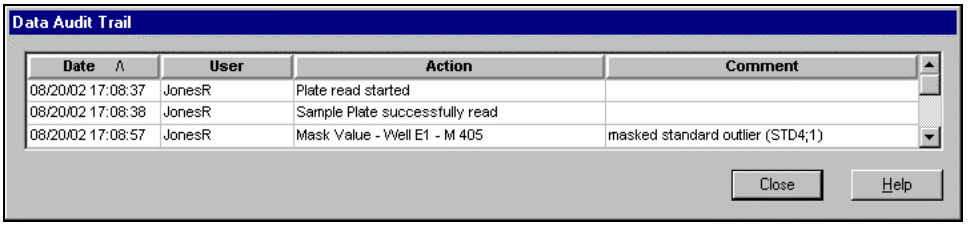
   KC4 automatically updates the **Data Audit Trail** to reflect the action:



To **mask** a well within any data file:

1. Open the desired file.
2. Within the Results window, click the **Data** drop-down arrow and select the desired data set. **Note:** When a well is masked, KC4 excludes its raw measurement value from further calculations.
3. Click the **Mask** box.
   - The System Administrator can **protect** the use of the Mask function. If you are trying without success to use this function, you may not be authorized to use it.
4. Click on a well in the grid to mask it (click again to unmask).
5. When the well(s) have been masked, click **OK** to recalculate results.

KC4 automatically updates the **Data Audit Trail** to reflect the action:



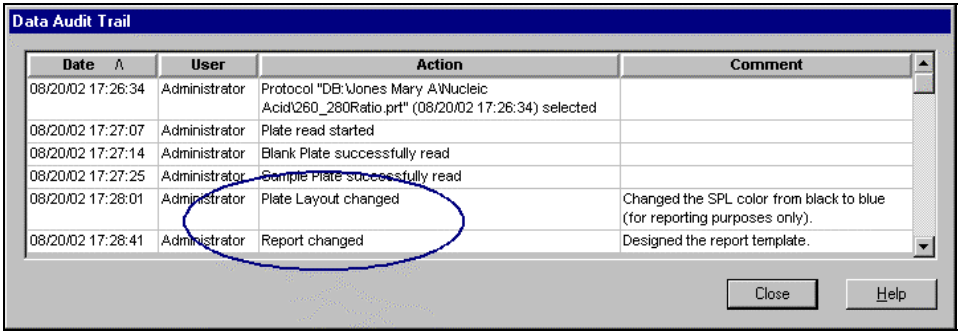
To **modify protocol parameters** within a GLB file:

1. Open the desired file.
2. Use the **Protocol** menu to select a parameter category to modify, such as Plate Layout, Single Plate Transformations, Report, and so on. Select Protocol|Advanced to see additional options including Cutoffs and Validation.

   Note: Reading parameters (such as filters/wavelengths and plate geometry) cannot be modified after a plate has been read.
3. Within the appropriate protocol parameter dialog, make the desired change and click **OK** to recalculate results.

   - The System Administrator can **protect** some or all protocol parameters from being modified. If you are trying without success to modify these parameters, you may not be authorized to do so.

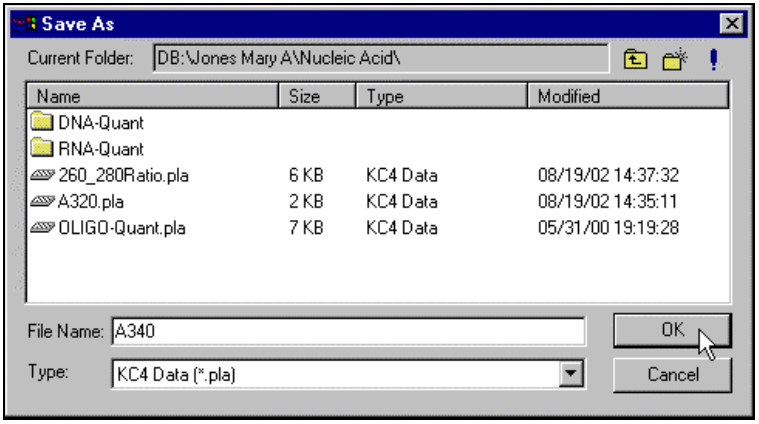
   KC4 *Signature* automatically updates the **Data Audit Trail** to reflect the action:



Saving Data Files

After creating or modifying a data file, save it by selecting Data|Save or Data|Save As. **Save** stores the data file under its current name. (If the file does not have a name, KC4 will ask you to enter one.) **Save As** copies the contents of an existing data file to a new data file with a different name.

Note: The layout of the Save or Save As dialog varies slightly depending on whether or not the Secured Protocol and Data File Storage option is enabled, but the functionality is the same. (In the following example, this option is *enabled*.)



- The **Current Folder** field or the **Save In** field (you will see one or the other) shows your current location within the overall folder structure.
  - If the drive is represented as **DB:**, this means that files are currently being stored in KC4's secure, shared-access database. If the drive is represented by a single letter, such as **C:** or **D:**, this means that files are stored individually on the computer's hard drive or on a network to which the computer is attached. See "Where and How is Plate Data Stored?" for more information.
- The default file name is NewPlate.
- Enter a new **File Name** and click OK to save it.
- Click to move up one folder level.
- Click to create a new folder.
- Click on a folder to open it and view its contents.
- Click the **Type** drop-down arrow to change the type of files being viewed. Options include:
  - KC4 Data (*.PLA)
  - KC4 Data+Protocol (*.GLB)
  - All Files (*.*)
- If an existing file name is entered, KC4 will provide a warning to prevent an accidental overwrite. Click **Yes** to continue with the overwrite, or **No** to rename the file before saving.

Closing Data Files

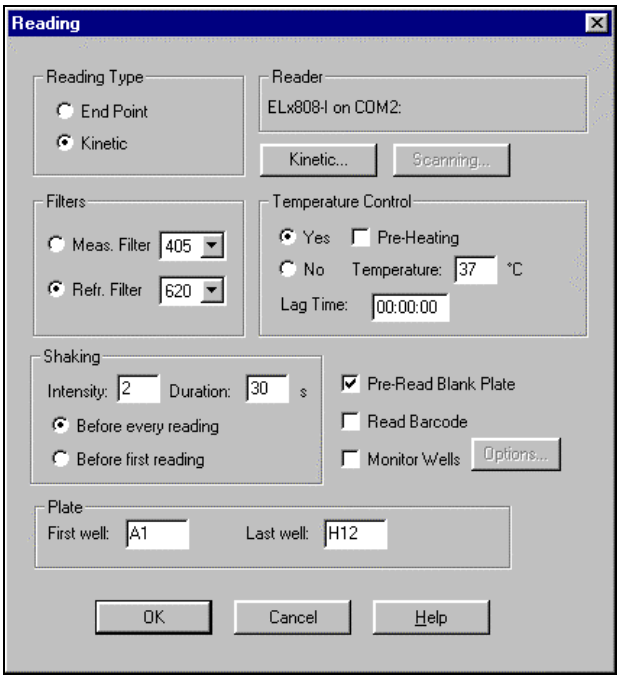
Select **Data|Close** to close the current data file. If the file has not already been named, the **Save As** dialog will appear requesting entry of a file name.

Note: Clicking in the upper right corner of the Results window does not close the data file.

Reading Plates

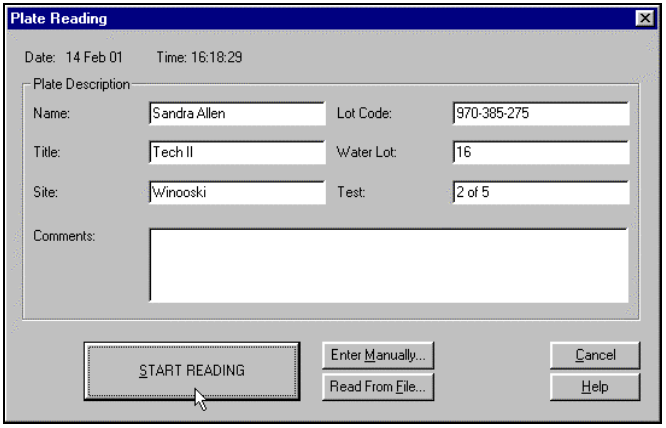
Once the new data file has been created (see "Creating a New Data File" on page 43), select **Data|Read Plate** to initiate a plate read, or to enter data manually or read data from a file.

If no protocol is open, a **Reading** parameters dialog appears. Parameters available within this dialog are reader-specific:



- Define the required **Reading** parameters and then click **OK** to continue.

  The **Plate Reading** dialog appears:



The **Plate Reading** dialog allows optional information and comments to be added to the plate. This information is filed along with the data for future reference.

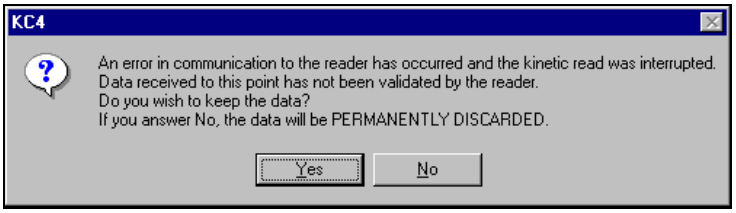
- (Optional) Enter data in the **Plate Description** fields, and enter **Comments.**

  Tip: The data entry field names can be customized via Protocol|Plate Description.

- Click **Start Reading** to initiate communication with the attached microplate reader. Following a successful plate read, the measurement values will be transferred to KC4 and displayed in the Results window.
- Click **Enter Manually** to enter plate data using the keyboard. See "Entering Plate Data Manually" on page 50 for more information.
- Click **Read From File** to read plate data from a stored file. See "Reading Plate Data From a File" on page 51 for more information.

Unintentional Termination of a Kinetic Read

If a kinetic read is unintentionally terminated, or if at the end of the read KC4 receives an error status from the reader, KC4 presents the following message:


KC4
An error in communication to the reader has occurred and the kinetic read was interrupted.
Data received to this point has not been validated by the reader.
Do you wish to keep the data?
If you answer No, the data will be PERMANENTLY DISCARDED.
Yes
No

As the message states, the read was interrupted and KC4 is providing the opportunity to save or discard the kinetic data received up to this point. The data is unvalidated, which means the reader did not (or could not) provide KC4 with the expected valid status at the end of the plate read.

The term "unintentional termination" applies to the following situations:

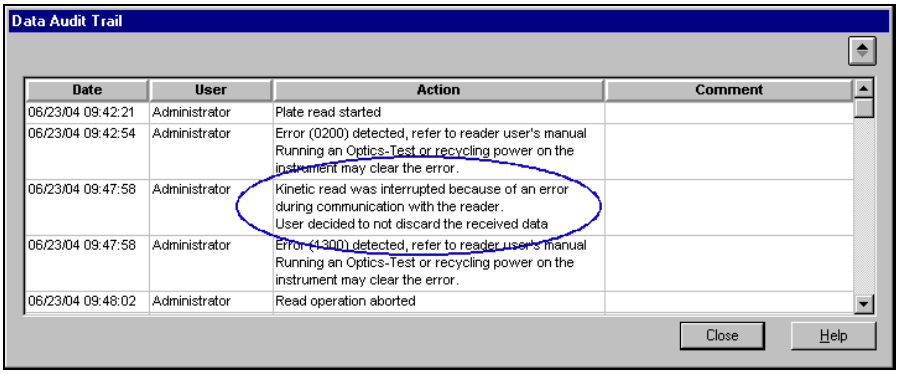
- The reader was turned off or the serial cable was disconnected during the read.
- KC4 received an error status string at the end of the kinetic read. **Note:** If the unvalidated data is saved after this error, the last kinetic point for each well is deleted due to its proximity to the error status.
- KC4 received an unexpected response from the reader or the reader stopped operating during the read (possibly due to a hardware error or an internal software error).
- MS® Windows™ experienced a communication error in the low level COM processes.

If the message shown above appears in KC4, take a moment to try to determine its cause. If the reader was inadvertently turned off or the serial cable was disconnected, the reader may have performed normally up to the point of termination, in which case the data would be valid. If the termination appears to have been induced by the reader, the data may not be valid. If a 4-digit error code is also supplied, consult the reader's Operator's Manual.

Within the message box:

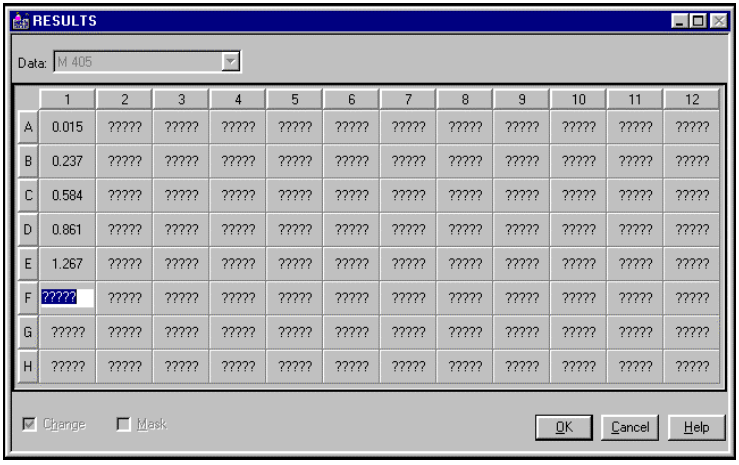
- If **NO** is selected, all data collected up to this point is permanently deleted and cannot be retrieved.
- If **YES** is selected, all data collected up to this point will be saved. It is strongly advised that the data be analyzed to determine its validity.

KC4 *Signature* users: If Yes is selected an event is logged to the Data Audit Trail, as demonstrated below:



Entering Plate Data Manually

KC4 provides the option to enter Endpoint and Kinetic plate data manually via the keyboard. Typically this information comes from other instruments or from previously acquired data.



- Use the up and down arrow keys to move from well to well in the matrix. When the down arrow is pressed from a well in the bottom row, the cursor automatically jumps to the well in the top row of the next column.
- Place the cursor over the desired well and enter the **OD** (or **RFU/RLU** if fluorescence / luminescence) value.
- Click **OK** to end the editing process and calculate results.
- For **multi-wavelength** reads, enter data for the first wavelength and click **OK.** For the remaining wavelengths, select the appropriate data set and click **Change** to enter values.
- For **kinetic** reads, enter data for Reading #1 and click **OK.** For the remaining readings, update the Reading # field and click **Change** to enter values.

  Note: With kinetic plate data, each reading number set must be incremented to mimic the kinetic plate reading cycle. The time base will be taken from the kinetic reading parameters defined in the protocol.

Reading Plate Data from a File

KC4 can read endpoint or kinetic data from an **ASCII Text file.** The file must be formatted properly, and the data must be delimited with acceptable separators.

To import Endpoint or Kinetic data:

- Examine the text file to see what character is used to **delimit** the data. Typical delimiters include semi-colon, comma, and tab.
- In KC4, select **System|Setup.** Set the **Import/Export Separator** so that it matches the character used within the text file.
- If desired, create or open a **protocol.**
- Select **Data|Read Plate|Read from File,** then select the desired file. If the file is formatted properly, KC4 will read the data then calculate results according to the protocol parameters.

Import File Format for an Endpoint Read

The file block size must correspond with the plate layout defined in the protocol. For example, a 96-well plate has 8 rows and 12 columns and a 24-well plate has 4 rows and 6 columns. The following example represents the 96-well plate format:

```
0.003;1.409;0.483;2.022;1.463;1.200;0.876;2.240;2.057;1.867;0.435;2.147
1.776;1.284;0.760;0.037;0.229;0.911;0.368;0.415;2.471;1.114;0.298;0.012
0.022;0.945;1.329;1.428;1.504;1.518;0.416;1.658;1.127;0.880;0.143;1.519
1.958;2.007;1.300;0.755;2.190;1.817;2.390;2.314;1.348;0.356;1.155;0.588
2.156;0.524;1.949;2.109;2.492;2.499;1.529;0.981;0.666;0.743;2.100;0.059
0.940;0.232;1.693;0.141;0.022;2.297;0.690;0.682;1.470;1.728;2.094;1.816
1.212;0.513;1.859;1.171;1.145;2.373;1.861;0.271;1.498;0.963;1.838;1.522
1.431;0.903;0.379;0.563;1.063;2.007;1.293;2.475;1.879;0.864;0.422;1.643
```

Import File Format for a Kinetic Read

Before importing a kinetic text file, create a protocol that specifies the exact number of kinetic reads that will be imported.

The first line in the text file can optionally contain a **time indicator.** This value will be stored in the protocol (overwriting protocol settings, if they are different), and used for any kinetic plotting.

If the file contains no time indicators then the time parameters defined via Protocol|Reading parameters will be used as defaults.

The following example represents the kinetic data set generated from 5 readings, in the 24-well format (to ensure a successful import of all five reads shown in this example, create a protocol and set the kinetic parameters so that the Reading Number is 5.):

```
Time=00:00:00
0.000;0.007;0.013;0.020;0.026;0.033
0.004;0.011;0.017;0.024;0.030;0.037
0.008;0.014;0.021;0.028;0.034;0.041
0.012;0.018;0.025;0.032;0.038;0.045
Time=00:00:30
0.000;0.026;0.053;0.079;0.105;0.132
0.016;0.042;0.068;0.095;0.121;0.147
0.032;0.058;0.084;0.110;0.137;0.163
0.047;0.074;0.100;0.126;0.153;0.179
Time=00:01:00
0.000;0.059;0.118;0.178;0.237;0.296
0.036;0.095;0.154;0.213;0.272;0.331
0.071;0.130;0.189;0.249;0.308;0.367
0.107;0.166;0.225;0.284;0.343;0.402
Time=00:01:30
0.000;0.099;0.197;0.248;0.284;0.356
0.056;0.195;0.194;0.295;0.322;0.381
0.171;0.188;0.231;0.318;0.390;0.401
0.167;0.216;0.275;0.337;0.403;0.499
Time=00:02:00
0.000;0.176;0.251;0.299;0.378;0.446
0.117;0.281;0.295;0.375;0.397;0.441
0.257;0.275;0.331;0.388;0.438;0.482
0.239;0.296;0.369;0.402;0.492;0.557
```

Importing Clarity Luminescence Data Files

Applies to Clarity™ Microplate Luminometer users only.

KC4 supports the importing of **Clarity results files (*.bti)** through its 'Read from File' feature when Clarity is the currently selected reader. You can, for example, import a data file containing raw luminescence data and then create a KC4 protocol to apply transformations, calculate curves, generate reports, and so on.

The following requirements must be met to ensure a successful import:

- The Clarity file must have a **.bti** extension.
- Before the .bti file is saved in the Clarity PC software, the **Displayed digits after period** field under Options|Visual Settings must be set to 0.
- If you wish to import a **.bdf** file, open it in the Clarity PC software, select File|Save As, and select the **.bti** file type.
- **Batch Protocols** must contain a maximum of 300 reads and have a total measurement time of less than 48 hours.
- If the file has a **measurement comment** associated with it (Measurement|Measurement Comment), the comment cannot contain any empty lines. For example, the following comment would cause the file import to fail because the second line is empty:

  (1st line) Measurement performed by Brian Smith

  (2nd line) <empty>

  (3rd line) Results approved by Sharon Jones

Notes on background measurement values:

If the Clarity file specified a background measurement and the 'Subtract instrument background' option was enabled, KC4 imports the subtracted values as well as the background value for informational purposes. This value can be viewed in KC4 by selecting Protocol|Reading when the data file is open.

If the Clarity file specified a background measurement and the 'Subtract instrument background' option was *not* enabled, KC4 imports the raw data values and the background value and then subtracts the background value from all wells.

Signing Data Files

KC4 *Signature* provides an option for users to permanently attach, or **sign,** their names to global data (GLB) files.

Notes:

- While KC4 does not permit users to sign plate data (PLA) files, if a plate data file and a protocol file are open at the same time, selecting Data|Sign will combine the two individual files into one signed GLB file.
- KC4 only supports file signatures when the **multi-user identification** feature is in use. See "Security" in *System Menu* for more information.

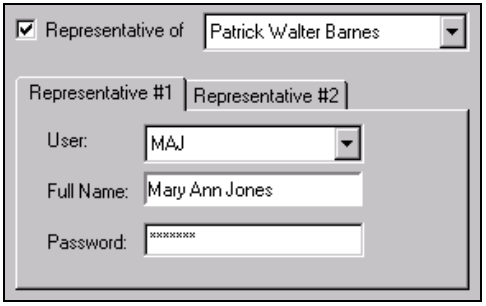
To sign a global data file:

1. Open the desired file (if it is not already open).
2. Select **Data|Sign** or click the Sign toolbar icon.

   The **New Data Audit Trail Signature** dialog will appear:

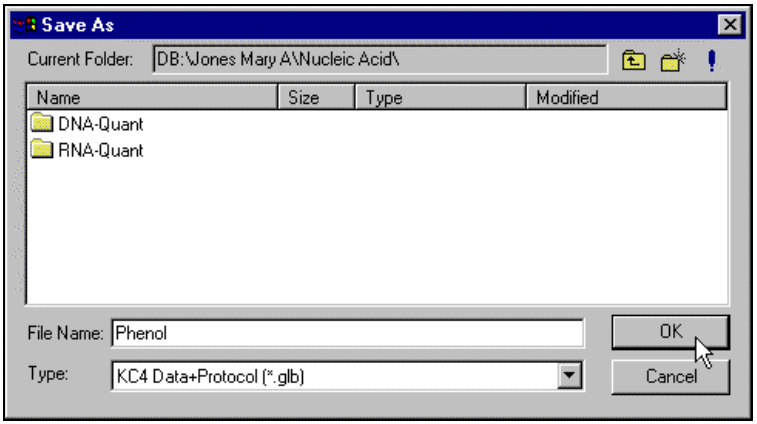
3. Click the **Reason** drop-down arrow and select the reason to be associated with this signature.
   - Signature Reasons are site-defined by the System Administrator under System|Security|Signature Reasons.

4. (Optional) If two users are signing on behalf of a third KC4 user, check the **Representative of** box and then choose a name from the drop-down list.

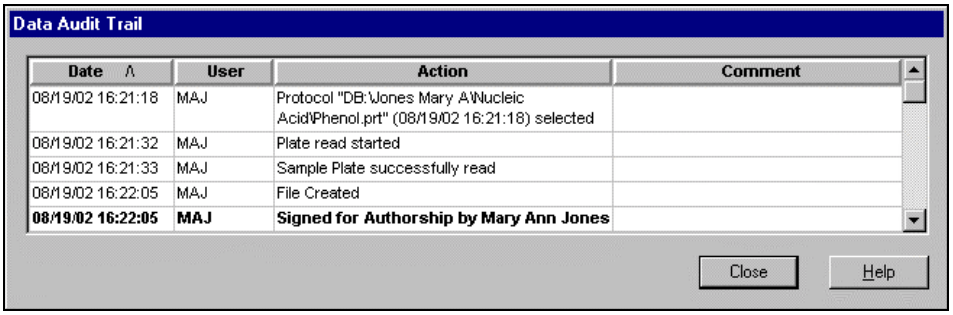


5. Enter or select your **User ID.**
   - If 'Representative Of' is checked, **two users** must enter or select their User IDs.
6. Enter your **Password.** The characters will not be visible on the screen.
7. Click **Sign & Save.**

   If the file has not yet been saved, a Save As dialog will appear:



8. Using the folder location tools provided, select the storage location.
9. Enter the desired **File Name** and click **OK.** The signature is applied immediately and KC4 logs the event in the **Data Audit Trail** (select Data|Audit Trail to view):



Append to Kinetic File

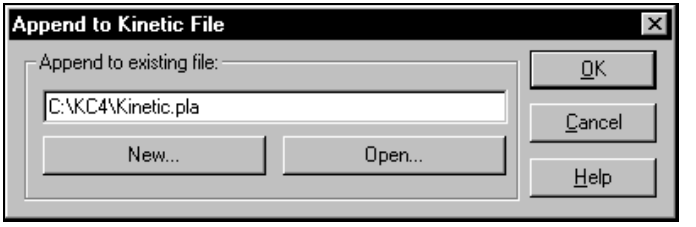
KC4 provides the option to build a kinetic file using individual endpoint read plates. The currently-open plate can be **appended** to the previously read plate. This feature allows plates to be read on a discontinuous time base, unlike pure kinetic readings.

- Alternatively, it is possible to append several **kinetic** readings to one file. If a kinetic reading sequence is halted (ex. to inject reagents) then it is possible to resume the kinetic reading and **append** the resumed data onto an existing file.
- The Append feature can be set to perform automatically after a plate read. See "Protocol Options" in *Protocol Menu* for more information.

To append a set of plate data to a kinetic file:

- When the plate read is complete, select **Data|Append to Kinetic File.**

  The **Append to Kinetic File** dialog appears:



  - The last opened plate file name appears as the default. Change the path and/or file name if desired.
  - Click **New** if this set of data represents the starting kinetic read (i.e., if subsequent reads will be appended to this one). If no file name has been entered, KC4 will produce the standard Save As dialog.
  - Click **Open** to search through the directory structure to locate the target file.
  - Click **OK** to continue. A dialog will appear requesting entry of a **Reading Time** relative to the first reading.

    The default value represents the elapsed time since the last plate read; enter a new value if required. **Note:** The time format is defined under System|Setup. For an interval greater than 99:59:59, set the time format to HHH:MM.

Exporting Plate Data

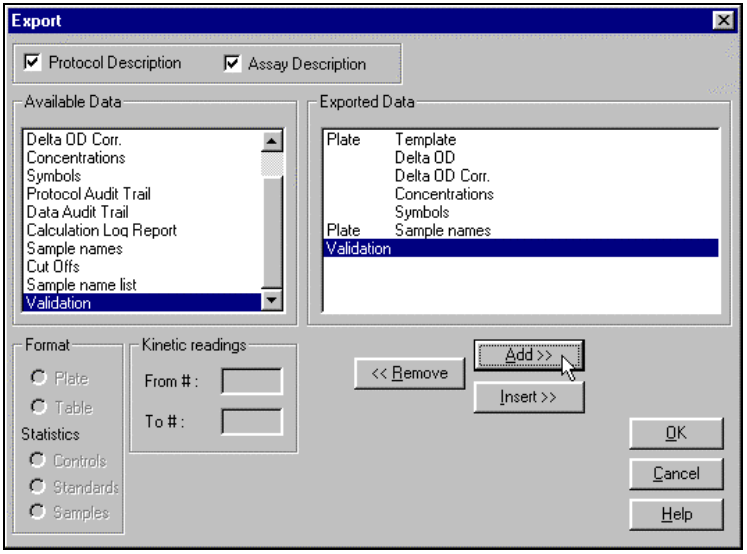
KC4 can **export** plate measurement data to a text file, a Microsoft® Excel spreadsheet, or a Microsoft® Word document. This section contains instructions for exporting to a text file only (using the KC4 export engine).

- See "Export" in *Protocol Menu* for more information on the available export engines.
- See *PowerReports* for more information on the Excel and Word exporting engines.

The export file format can be defined within a protocol (under **Protocol| Export**), or after the plate is read (by selecting **Data|Export).** The text delimiter used during the export is selected under **System|Setup.**

To **export** data:

- When the plate read is complete, select **Data|Export.**
- If export parameters are defined in the protocol, KC4 requests the entry of a **file name.** Enter the file name and then click **Save** to export the data. Data exported to a text file can be used by any other program with the ability to read an ASCII file.
- If export parameters are *not* already defined in the current Protocol, an Export template appears. The example below shows the template used to export data to a text file:



- Select the desired data sets and then click **OK** to begin the export. KC4 requests the entry of a **file name.** Enter the file name then click **Save** to export the data. Data exported to a text file can be used by any other program with the ability to read an ASCII file.
  - Regardless of the Secured Protocol and Data File Storage setting, data is exported as an individual .txt file outside the shared-access database.
  - See "Export" in *Protocol Menu* for information on customizing the export template.

Sample Export Text File

```
[ProtocolDescr]
Name=C:\KC4 3.4\Sample Export.prt
Modified=No
[ReadingParameters]
PlateSize=4,6
PlateType=24 WELL PLATE
Wells=A1-D6
ReadBlankPlate=No
PathlengthCorrection=No
ReadBioCell=No
MonitorWell=No
Filter1=405
Calibrate=No
[DataDescription]
Name=C:\KC4 3.4\Sample Export.pla
Barcode=123-ABC-7890
ReadingDate=03/14/01 13:19:16
ReadingType=Reader
Temperature=
PromptTitles=Prompt #1:;Prompt #2:;Prompt #3:;Prompt #4:;
Prompt #5:;Prompt #6:
Prompts=;;;;;
Comment=This is a sample.
ReportTime=03/14/01 13:21:41
[Plate: Template]
;1;2;3;4;5;6
A;STD1:10.000;SPL1;SPL3;SPL5;SPL7;SPL9
B;STD2:20.000;SPL1;SPL3;SPL5;SPL7;SPL9
C;STD3:30.000;SPL2;SPL4;SPL6;SPL8;SPL10
D;STD4:40.000;SPL2;SPL4;SPL6;SPL8;SPL10
[Plate: M 405]
;1;2;3;4;5;6
A;0.006;2.818;0.967;4.044;2.925;2.399
B;0.751;4.480;4.114;3.733;0.871;4.295
C;1.532;2.568;1.520;0.075;0.457;1.822
D;2.737;0.829;4.943;2.228;0.595;0.023
[Plate: Concentrations]
;1;2;3;4;5;6
A;11.065;>41.500;21.774;>41.500;>41.500;37.731
B;19.367;>41.500;>41.500;>41.500;20.704;>41.500
C;28.070;39.614;27.936;11.834;16.091;31.302
D;41.498;20.236;>41.500;35.826;17.629;11.255
[Plate: Symbols]
;1;2;3;4;5;6
A;NEG;OUT+;EQV;OUT+;OUT+;POS
B;EQV;OUT+;OUT+;OUT+;EQV;OUT+
C;POS;POS;EQV;NEG;NEG;POS
D;POS;EQV;OUT+;POS;NEG;NEG
```

Printing Reports

After plate measurement data has been acquired and calculations are complete, KC4 can generate a user-customizable set of results reports. Reporting options are selected and customized within each protocol by selecting **Protocol|Reports.**

Print

Select **Data|Print** or click the appropriate toolbar icon to print the reports selected in the protocol.

Print Preview

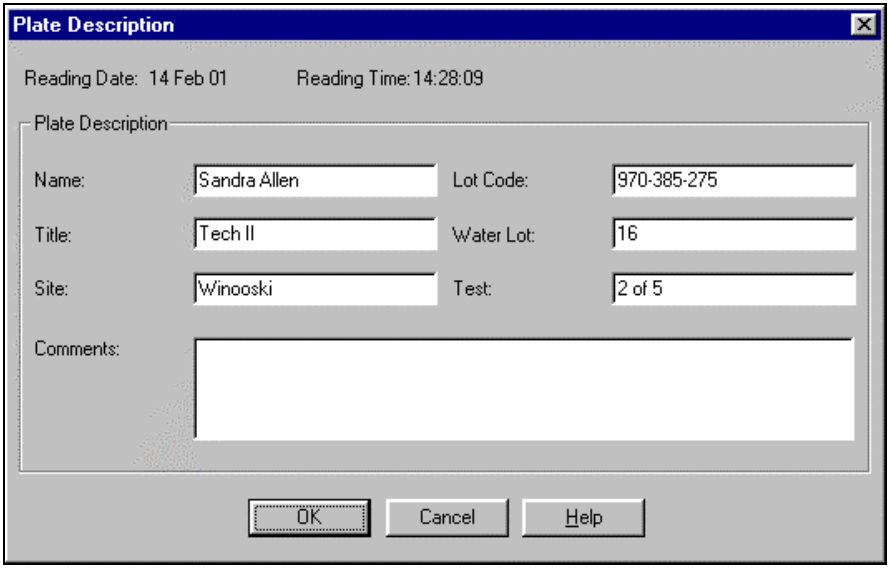
Select **Data|Print Preview** to see what the report will look like before it is printed.

Print Setup

To change the printer, paper size or source, or page orientation, select **Data|Print Setup** to access a standard Print Setup dialog.

Plate Description

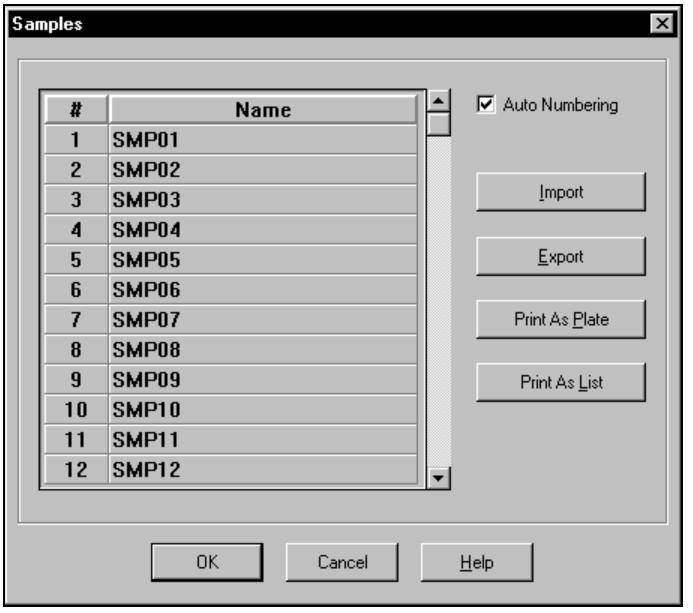
The **Plate Description** dialog appears just prior to each plate read, to provide the optional entry of plate-specific information.



- The **Reading Date** and **Time** are automatically logged when the plate is read. Enter additional data as desired and then click **OK** to continue with the plate read.
  - Data entry field names can be customized by selecting **Protocol|Plate Description.**
- The information contained within this dialog is automatically stored in the data file when **Data|Save** is selected.

Samples

Select **Data|Samples** to assign names to the sample groups previously defined under Protocol|Plate Layout.



Each sample ID can be entered **manually,** or the **Auto Numbering** feature can be used to automatically increment ID suffix numbers.

- The ID entered in cell #1 will be assigned to every replicate in Sample group one, the ID in cell #2 will be assigned to every replicate in Sample group two, and so on.
- Up to 20 alphanumeric characters can be used for each ID.

  Note: If a sample name cannot be **printed** in its entirety, it is truncated and the character '\' is added at the beginning of the name to indicate that the name has been truncated. For example: '\LE001', '\LE002', etc.
- Sample IDs can be entered using a **barcode scanner** attached to the computer. To do this, place the cursor in cell #1 (or any empty cell). Scan the item with the barcode scanner. The first 12 characters of the ID will appear in KC4. Move the cursor down to the next empty cell, then scan the next item.

Auto Numbering

To automatically number the sample IDs sequentially:

- Check the **Auto Numbering** box.
- In cell #1, type the identifying name and starting number (e.g., Smp1) for Sample group #1. Leave the cursor in cell #1.
- Press the **down arrow** through as many cells as there are sample groups on the plate. KC4 will automatically increment the suffix number (Smp2, Smp3, and so on).

Import Sample Names

The **Import** option allows you to import a sample name list from a text file. Click **Import** and then locate and select the file to import.

- The import file must contain one name label per line.
- The number of IDs in the file should equal the number of samples as defined under **Protocol|Plate Layout.**

  If there are more IDs in the text file than samples defined on the plate, the extra IDs at the end of the file will remain unused.
- If a Sample ID in the text file exceeds 12 characters, only the first 12 characters will be imported.

Export Sample Names

The **Export** option allows you to export the generated identification labels to a text file for future use. Enter the Sample IDs as required and then click **Export** to specify the export file name. The export file will contain one name label per line.

Printing Sample IDs

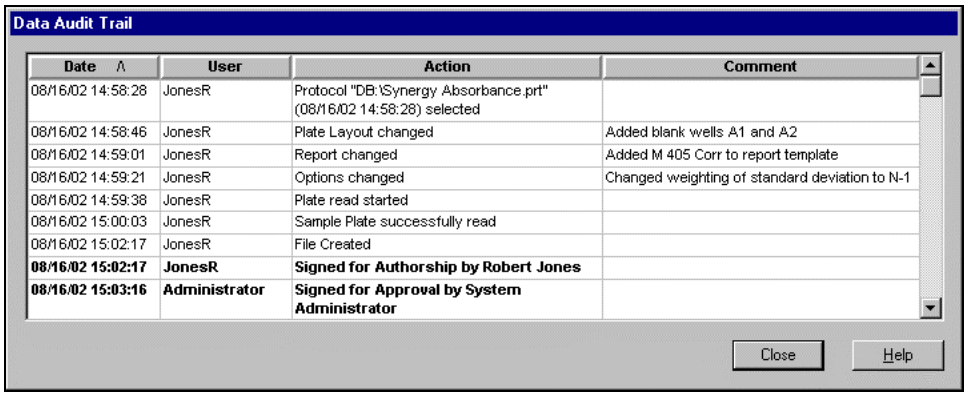
The **Print** options allow you to print the Sample IDs in a plate or list format.

- Click **Print As Plate** to print the Sample IDs in a matrix format corresponding with the plate format selected in the protocol. The plate must be configured via Protocol|Plate Layout prior to using this feature.
- Click **Print As List** to print the Sample IDs in a list format. One Sample ID will be printed on each line.

Data Audit Trail

Every data file (plate and global) has a **Data Audit Trail** associated with it. This audit trail automatically records **events** performed on the file since the file was created. Each event records an **action** such as "Sample Plate read" or "Mask Value - <well ID>" and includes a date and time stamp, the ID of the user logged in at the time (if applicable), and any comments that were entered by the user when the action was performed. The audit trail cannot be edited and it remains a permanent part of the file for the life of that file (that is, if the file is deleted, the audit trail is also deleted).

- With KC4 v3.4, only Change, Mask, Read, and Re-Read (delete initial values) actions are logged in the audit trail. A file's audit trail cannot be viewed from within KC4 but it can be added to report and export templates.
- With KC4 *Signature*, Change, Mask, and many other actions may be logged to the audit trail. A file's audit trail can be added to report and export templates, and can also be viewed by selecting **Data|Audit Trail:**



  - Events are sorted by date/time stamp and include the ID of the user logged in at the time, the action taken, and any comments that were entered by the user when the action was performed.
  - The contents of the data audit trail can be copied to the Clipboard by highlighting the desired cells and pressing Ctrl+C.
  - The contents of the data audit trail **cannot** be edited or deleted.

Edit Menu

Editing Tools

KC4 provides some editing tools for working with data in KC4. The two most commonly used tools are **Copy** to copy selected data to the clipboard, and **Undo** to remove the most recent unsaved change. Availability of editing tools depends on the current working environment; for example, the Cut and Paste options are not available within any measurement data grids or audit trail tables, but they are available within data entry fields (such as formulas).

Editing tools are available through the Edit menu, by clicking the right mouse button, or by pressing the shortcut key combination, and they can include:

- **Cut (Ctrl+X):** Deletes the selected data from KC4 and moves it to the clipboard. This function is not available within measurement data grids or audit trail tables.
- **Copy (Ctrl+C):** Copies the selected data from KC4 to the clipboard without deleting it. This function is available within measurement data grids, audit trail tables, and data entry fields. Note: The values copied to the clipboard are separated by the character selected under System|Setup|Formats.
- **Paste (Ctrl+V):** Pastes data from the clipboard into the current KC4 location (wherever the cursor is placed). This function is not available within measurement data grids or audit trail tables.
- **Delete (Delete key):** Deletes the selected data from KC4 without moving it to the clipboard. This function is not available within measurement data grids or audit trail tables.
- **Select All:** Selects (highlights) all text within the current field or cell. For example, place the cursor in a formula cell within the Single Plate Transformation dialog, click the right mouse button and click Select All to select the entire formula.
- **Undo (Ctrl+Backspace):** Erase the last (unsaved) change made and restore any items that had been modified by that last change. For example, if within the Plate Layout dialog you mistakenly applied Sample well identifiers atop existing Standard well identifiers, choosing Undo will restore the Standard wells.

Protocol Menu

Overview

A **Protocol** contains instructions for controlling a microplate reader and for analyzing and reducing plate measurement data. Protocol parameters can be stored in a protocol file (which has a .PRT extension) or in a global data file (which has a .GLB extension).

The topics in this chapter describe how to create and customize protocols. Customizable protocol parameters include the following:

- Detection method (Absorbance, Fluorescence, or Luminescence)
- Reading type (Endpoint, Kinetic, or Spectrum)
- Read mode (Normal, Rapid, or Sweep)
- Read/Dispense parameters (for instruments with fluid injection)
- Wavelength selection
- Linear and area scanning
- Plate type and reading zone selections
- Blank plate / well subtraction
- Pathlength correction
- Well monitoring
- Kinetic, spectrum, and scanning analysis
- Plate layout
- Single plate transformations
- Multi plate transformations
- Curve-fitting parameters
- Validation criteria
- Cutoff criteria
- Reporting and exporting options

Working With Protocols

This section provides instructions for creating, modifying, saving, and accessing protocols.

Where and How Are Protocols Stored?

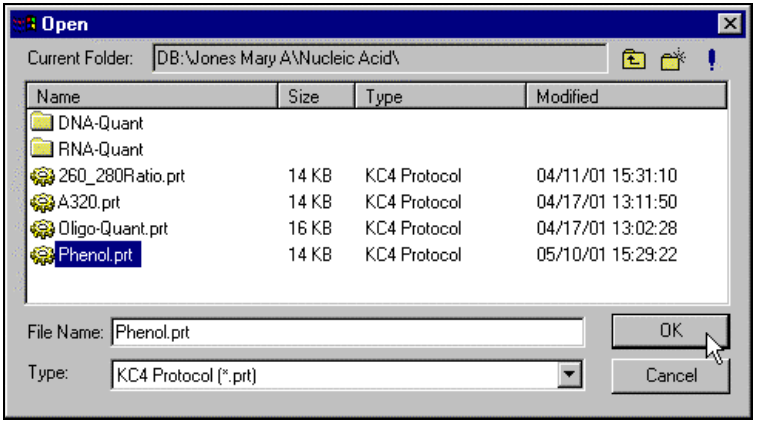
KC4 stores protocols using a proprietary file format and the .PRT extension. File storage options vary depending on site requirements and the current KC4 configuration.

- Files may be stored individually on the computer's hard drive, on a CD or diskette, or on a network to which the computer is attached. This is the storage method that users of KC4 version 3.0x and earlier are familiar with. Windows Explorer or a similar application can be used to view the file names and their locations, and to move, copy, rename, and delete files.
- (KC4 *Signature*) Files may also be stored inside a secure, shared-access database. This database, named KCShared.mdb, can be stored on a user's computer or on a shared-access computer. KC4 provides a special file maintenance window for viewing the file names and their locations, and to move, copy, rename, and delete files.

Opening Existing Protocol Files

Select **Protocol|Open** to open a previously saved protocol file. (If the Open option is not available in the Protocol menu, close the plate data file.)

Note: The layout of the **Open** dialog varies slightly depending on whether or not the Secured Protocol and Data File Storage option is enabled, but the functionality is the same. (In the example below, this option is *enabled*.)



- The **Current Folder** field or the **Look In** field (you will see one or the other) shows your current location within the overall folder structure.
  - If the drive is represented as **DB:**, this means that files are currently being stored in KC4's secure, shared-access database. If the drive is represented by a single letter, such as **C:** or **D:** this means that files are stored individually on the computer's hard drive or on a network to which the computer is attached. See "Where and How Are Protocols Stored?" for more information.

- Click to move up one folder level.
- Click on a folder to open it and view its contents.
- Click on a file and click **OK** to open it.
- Click to update the view to show any folders/files that may have been added by another user after you opened this dialog.

Upon opening a protocol, KC4 evaluates it to determine (1) if it was created with an earlier version of KC4, and (2) which reader model it was created for.

- If the protocol was created using an earlier version of KC4, the following message appears: "This file has been created with a former version of KC4. It is going to be converted. As a precaution, please verify after conversion that all of its parameters have been properly recovered. Then save the converted file." KC4 will not automatically save the converted file; it is your responsibility to verify and then save the file either under its existing name or a new one.
- If the protocol's reader model does not match the one currently selected under System|Readers, the following message appears: "Protocol file is not compatible with the current reader. The current reader will be changed." Click OK to open the protocol and allow KC4 to update the current reader selection under System|Readers, or click Cancel to return to the main view without opening the protocol.

  If you click **OK,** KC4 will set the 'current reader' to the reader that was selected when the protocol was created. If a different reader is then selected from the System menu, KC4 will delete the *reading parameters* of the open protocol, but will retain the data reduction steps of the protocol. It will also set the protocol name to "New Protocol.prt". The reading parameters must be re-entered and the data reduction steps (i.e. data in for curve fits, transformations, etc.) checked for correct input. The protocol can then be saved under a similar or new name.

  Note: If the attached reader does not match the one KC4 is expecting, the messages *INCORRECT READER MODEL CONNECTED* or *NO READER RESPONSE (TIMEOUT)* and then *Error when getting wavelength table from the reader* may appear. KC4 will proceed and change the reader under System|Readers but you will have to attach the correct reader before running the protocol.

When the protocol is open, KC4's title bar changes to show the file name. Use the Protocol menu or the toolbar icons to modify or view the protocol's parameters.

Creating New Protocols

Important: *Before* creating a new protocol, select System|Readers and set the Current Reader to the appropriate target reader model. The reader does not have to be connected to the computer when you are creating or modifying protocols.

Clarity™ Microplate Luminometer users: See page 77 for information on creating protocols.

To create a new protocol, begin by doing one of the following to enable the options under the Protocol menu:

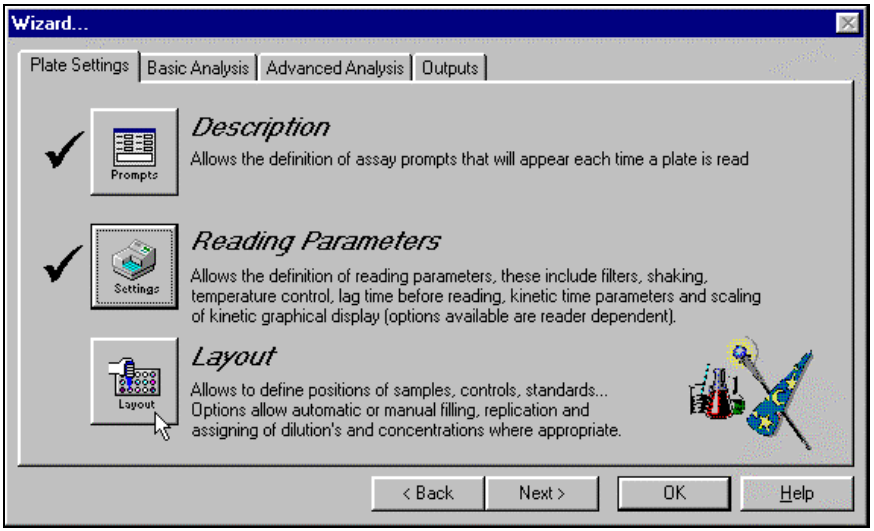
- Close any open plate data files and then select **Protocol|New.**
- If the Preferred Data File Format is set to .GLB, select **Data|New** and then choose **Empty Protocol.**

The next step is to define the protocol parameters required by your assay to control the reader and perform data reduction. Do this by opening the Protocol menu and selecting each option in turn: Reading, Kinetic Analysis, Plate Layout, and so on. Select 'Advanced' to see additional options including Cutoffs and Validation. The major headings in the remainder of this chapter are aligned with the Protocol menu options; refer to these sections for further instruction.

You may not need to use all of the options, but you must at a minimum define the Reading parameters. For more information see "Defining Reading Parameters" later in this chapter.

Protocol Wizard

As an alternative to using the Protocol Menu to create a new protocol, KC4 provides a **Wizard** to guide you through the process. Protocol parameters are conveniently categorized for easy selection and intuitive setup. Click the Wizard toolbar button or select **Protocol|Wizard** to begin.


Wizard...
Plate Settings
Basic Analysis
Advanced Analysis
Outputs
Prompts
Description
Allows the definition of assay prompts that will appear each time a plate is read
Settings
Reading Parameters
Allows the definition of reading parameters, these include filters, shaking, temperature control, lag time before reading, kinetic time parameters and scaling of kinetic graphical display (options available are reader dependent).
Layout
Layout
Allows to define positions of samples, controls, standards... Options allow automatic or manual filling, replication and assigning of dilution's and concentrations where appropriate.
< Back
Next >
OK
Help

- Click on a **button** (e.g. Settings) to define related parameters. KC4 displays a check mark (✓) next to each category after it is accessed.
  - You do not have to define the parameters associated with every button. At a minimum, however, you should define the Reading Parameters under the Plate Settings tab.
- Click on a **tab** (e.g. Basic Analysis) to see more buttons.
- Click **< Back** or **> Next** to switch between tabs.
- Click **OK** to close the Wizard.

Modifying Protocol Parameters

To modify protocol parameters within an existing protocol (.prt) or global data (.glb) file, begin by opening the desired file. Once the file is open, use the Protocol menu or the toolbar buttons to select a parameter category to modify, such as Reading, Plate Layout, Single Plate Transformations, and so on.

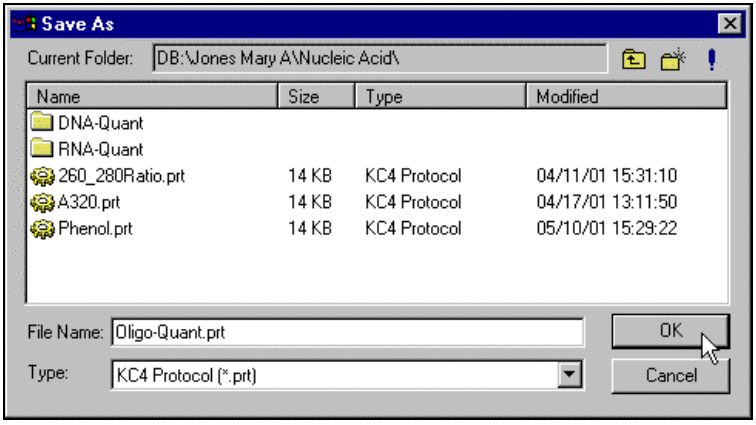
Notes:

- Select Protocol|Advanced to see additional menu options, including Multi Plate Transformations, Cutoffs, and Validation.
- When a file is being modified, an asterisk appears next to its name in the title bar as a reminder to save the changes: [Oligo-Quant.prt*]
- With KC4 *Signature*, KC4 automatically logs an event to the file's **audit trail** any time a change is made.

Saving Protocol Files

After creating or modifying a protocol file, save the information by selecting Protocol|Save or Protocol|Save As. **Save** stores the protocol under its current name. (If it does not have a name, KC4 will ask you to enter one.) **Save As** copies the contents of an existing protocol to a new protocol with a different name.

Note: The layout of the Save or Save As dialog varies slightly depending on whether or not the Secured Protocol and Data File Storage option is enabled, but the functionality is the same. (In the following example, this option is *enabled*.)

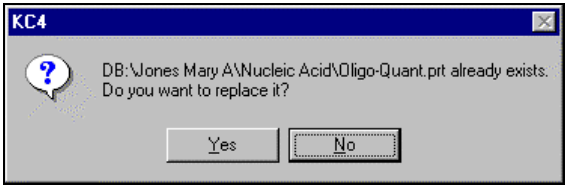


- The **Current Folder** field or the **Save In** field (you will see one or the other) shows your current location within the overall folder structure.
  - If the drive is represented as **DB:**, this means that files are currently being stored in KC4's secure, shared-access database. If the drive is represented by a single letter, such as **C:** or **D:** this means that files are stored individually on the computer's hard drive or on a network to which the computer is attached. See "Where and How Are Protocols Stored?" for more information.

- The default file name is NewProtocol.prt
- Enter a new **File Name** and click OK to save it.
- Click to move up one folder level.
- Click to create a new folder.
- Click on a folder to open it and view its contents.
- If an existing file name is entered, KC4 will provide a warning to avoid an accidental overwrite. Click **Yes** to continue with the overwrite, or **No** to rename the file before saving.

Closing Protocol Files

To close a protocol file, select **Protocol|Close.** If the protocol is newly created, or if modifications were made to an existing protocol, KC4 provides an option to save:



- Click **Yes** to save the new or modified information and close the protocol.
- Click **No** to close the protocol without saving changes.
- Click **Cancel** to close this dialog and return to the open protocol. The new or modified information is retained but not yet saved.

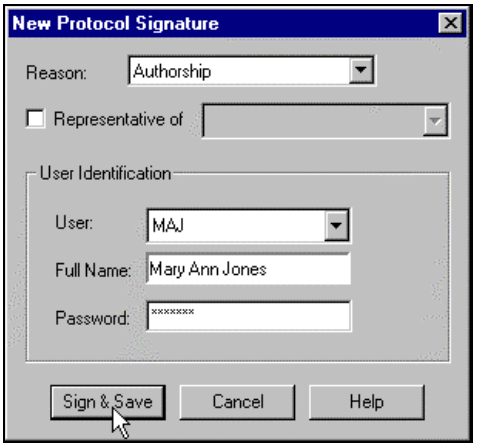
Signing Protocol Files

KC4 *Signature* provides an option for users to permanently attach, or **sign,** their names to protocol files.

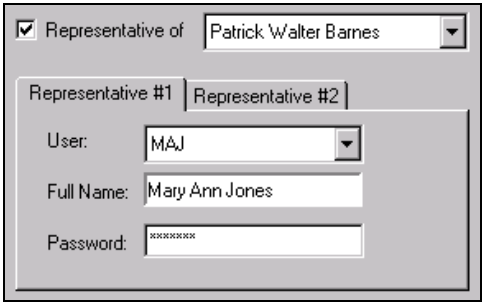
Note: KC4 only supports file signatures when the **multi-user identification** feature is in use. See "Security" in *System Menu* for more information.

To sign a protocol file:

1. Open the desired file (if it is not already open).
2. Select **Protocol|Sign.** The **New Protocol Signature** dialog will appear:

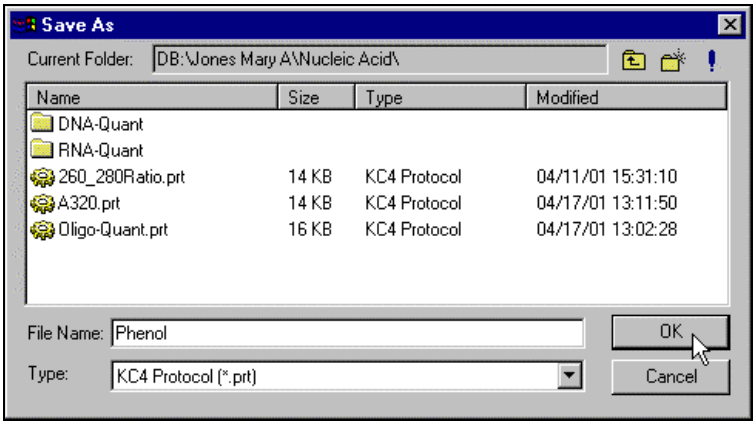


3. Click the **Reason** drop-down arrow and select the reason to be associated with this signature.
   - Signature Reasons are site-defined by the System Administrator under System|Security|Signature Reasons.
4. (Optional) If two users are signing on behalf of a third KC4 user, check the **Representative of** box and then choose a name from the drop-down list.



5. Enter or select your **User ID.**
   - If 'Representative Of' is checked, **two users** must enter or select their User IDs.
6. Enter your **Password.** The characters will not be visible on the screen.
7. Click **Sign & Save.**

If the file has not yet been saved, a Save As dialog will appear:



8. Using the folder location tools provided, select the storage location.
9. Enter the desired **File Name** and click **OK.** The signature is applied immediately and KC4 logs the event in the **Protocol Audit Trail** (select Protocol|Audit Trail to view):

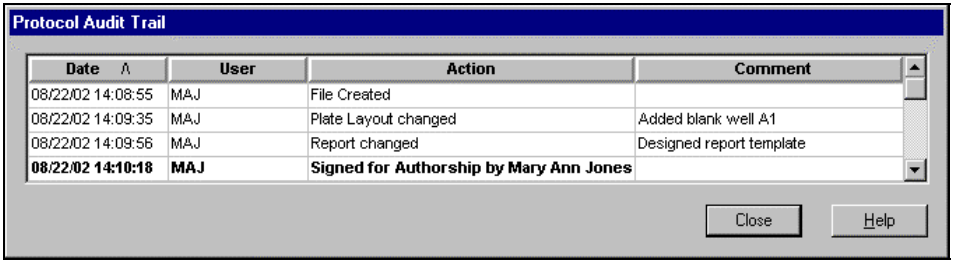
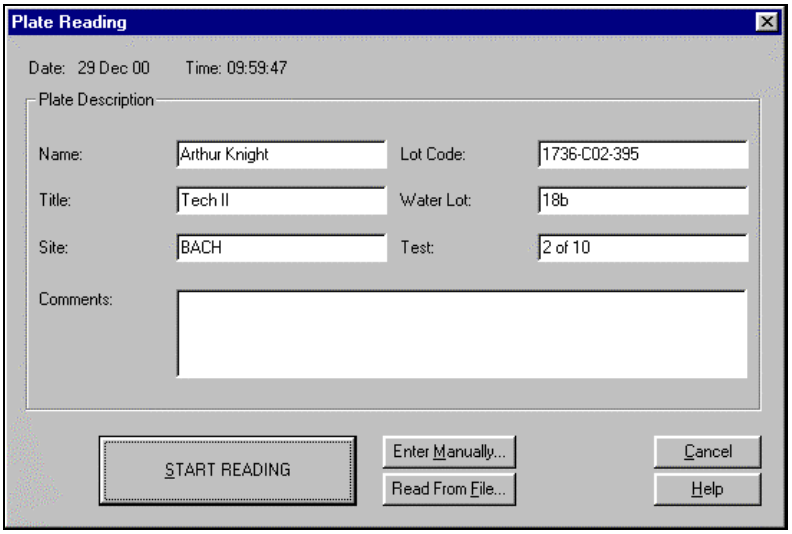


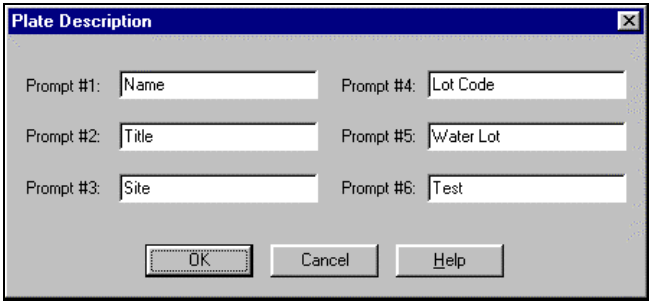
Plate Description

The **Plate Description** dialog appears just prior to each plate read, to provide the optional entry of plate-specific information.



This dialog gathers information to be stored in the plate file along with the measurement data.

KC4 allows you to define up to six free-text fields to appear in this dialog (they are shown above as Name, Title, Site, Lot Code, Water Lot, and Test). Select **Protocol|Plate Description** to customize the names of these fields.



- Text entered here substitutes the **Prompt #** text seen in the Data|Plate Description and Data|Plate Read dialogs.
- To customize a prompt, simply overwrite the default text in the **Prompt #** field.
- Enter up to 15 alphanumeric characters in each field.
- If a field is left empty, the prompt will be kept out of the Plate Description dialog.

Defining Reading Parameters

The configuration of the **Reading** parameters controls plate reading and measurement data collection. The availability of Reading parameters varies depending on the reader type currently selected under **System|Readers.** To define Reading parameters, select **Protocol|Reading** or click the **Settings** toolbar button to open the Reading dialog.

The following pages are organized by reader model; locate your model to find the basic instructions for defining Reading parameters. To learn more about a particular parameter, search through the *Reading Parameters Reference*, which is organized alphabetically.

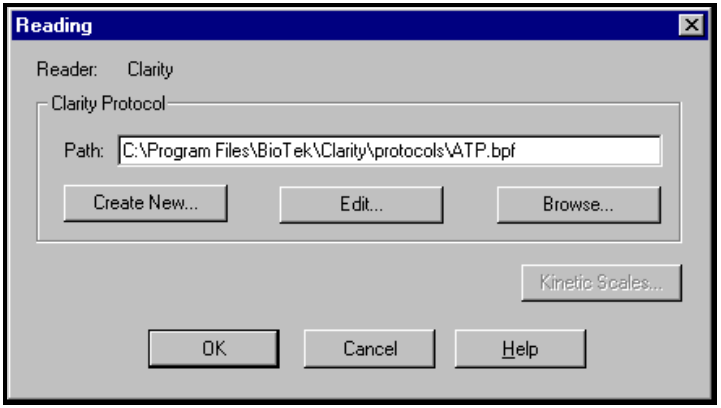
Clarity™

When KC4 is used to control the Clarity Microplate Luminometer, two protocols are required: a **Clarity protocol file** to specify the reading parameters and a **KC4 protocol** to specify the data reduction parameters. The KC4 protocol references the Clarity protocol file.

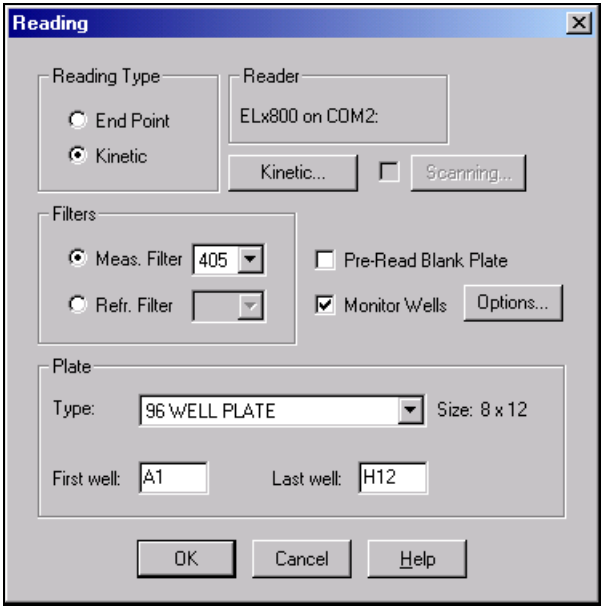
Clarity protocol files have a **BPF** extension and they are typically (but not necessarily) stored on the PC in the **C:\Program Files\BioTek\Clarity\protocols** folder. BPF files cannot be stored in KC4's shared database.

Clarity protocol files can be created and edited through either the Clarity PC Software or KC4. In either case, information on Clarity's reading parameters can be found in Chapter 4 of the **Clarity Operator's Manual** under the "Parameter Input" heading.

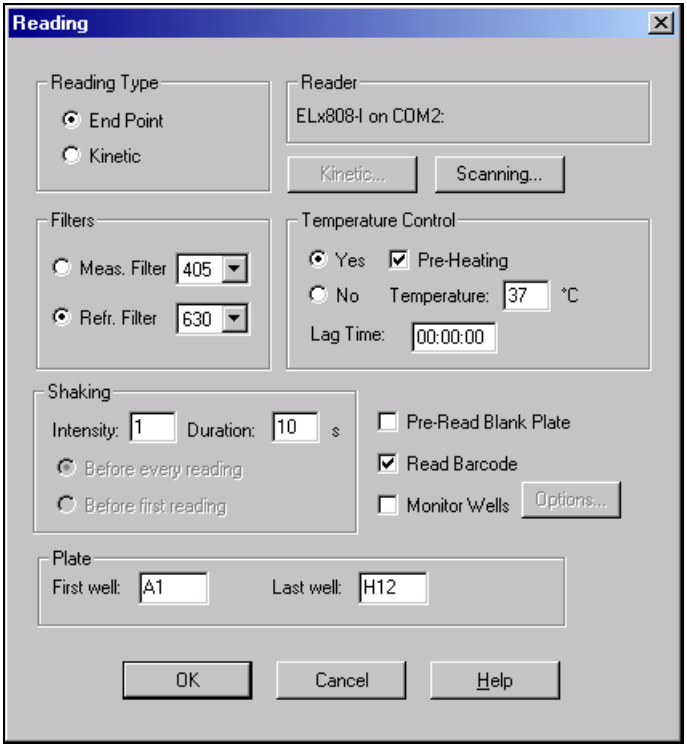
To create/edit/reference a Clarity protocol file within a KC4 protocol, make sure that Clarity is set as the current reader (System|Readers) and then select **Protocol|Reading.** The **Reading** dialog will appear, as shown below:



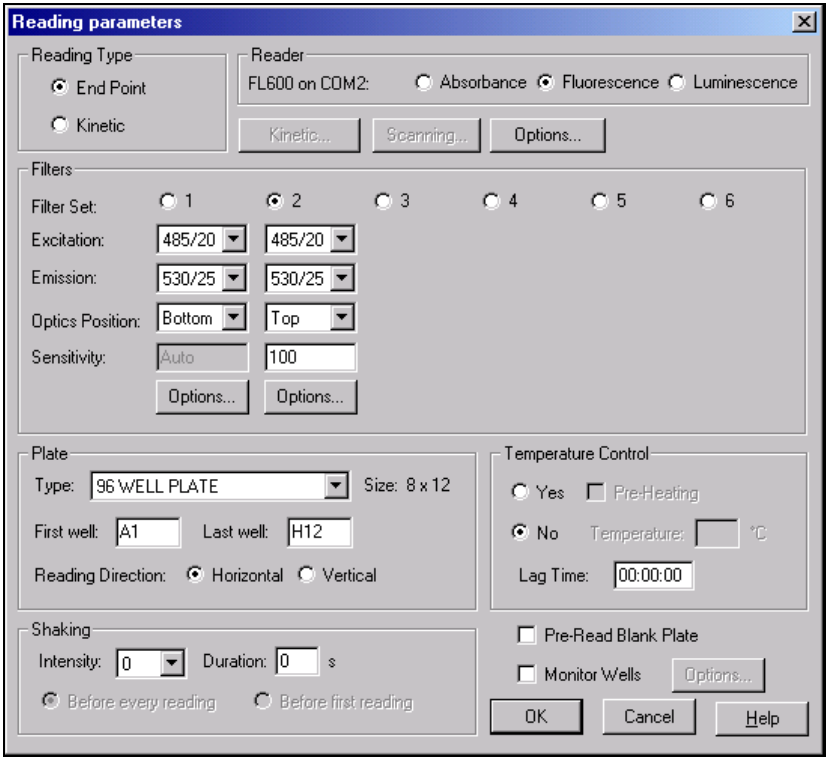
- To create a new Clarity protocol file, click **Create New.** The **Create Protocols** dialog will appear. Select a protocol type and click **Create** to advance to the parameter input series of dialogs.
- To search for and open an existing Clarity protocol file, click **Browse.** The file's extension must be .BPF. Highlight the file and click **Open.** The file's name and location will appear in the **Path** field.
- To edit reading parameters for the currently selected file, click **Edit.** The **Protocol Settings** dialog will appear. Make the necessary changes and then click **Save** to store the settings using the current file name, or **Save As** to store the settings using a new file name.
- (Optional) For Fast Kinetic protocols, click the **Kinetic Scales** button to adjust the min/max t and min/max y scales for curve displays.
- When finished, click **OK** to save changes or **Cancel** to discard them. You can now define data reduction parameters via the **Protocol Menu** (Plate Layout, Transformations, Cutoffs, Reports, etc.) or run the protocol by selecting **Data|Read Plate.**



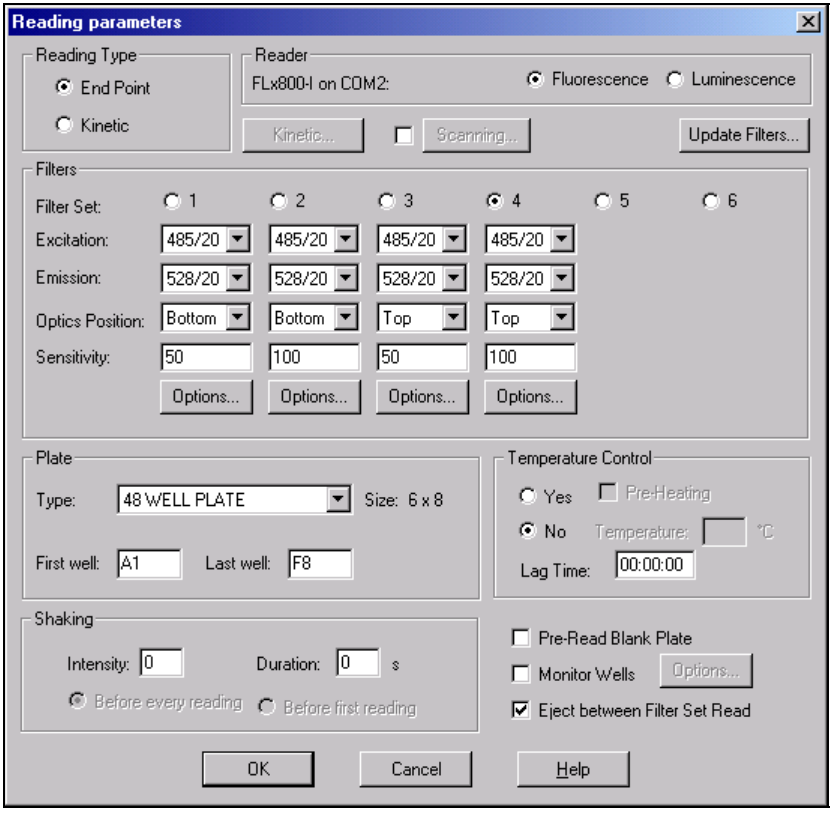
1. Select a **Reading Type** of Endpoint or Kinetic.
2. Select the **filter(s)** to be used.
3. Select a **Plate Type** and define the **First** and **Last Wells.**
4. For a **kinetic** protocol, click the Kinetic… button to define the read time and interval.
5. For an endpoint **area scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
6. (Optional) Select **Pre-Read Blank Plate.**
7. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
8. Click **OK** to verify the parameters and close this dialog.



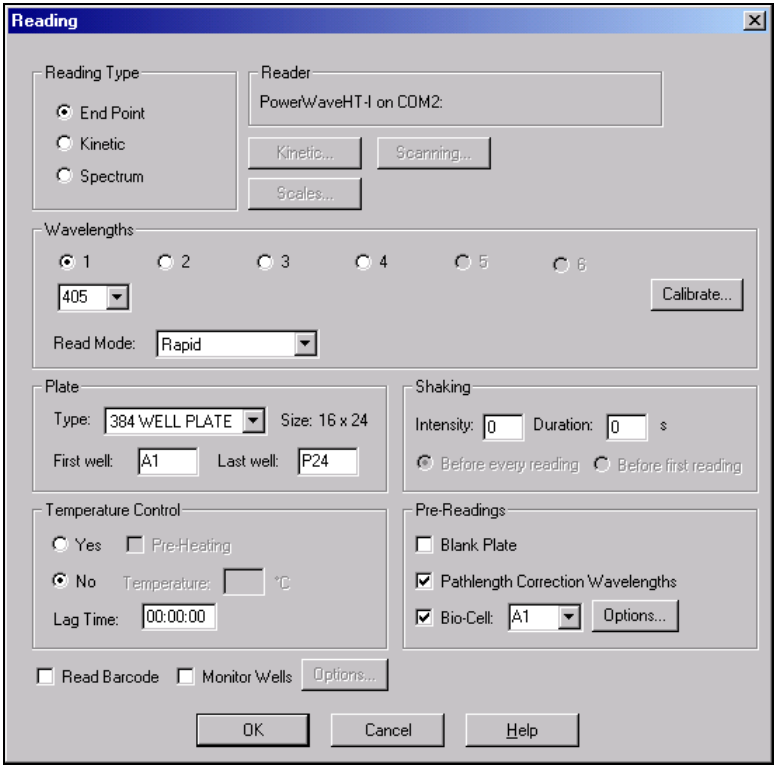
1. Select a **Reading Type** of Endpoint or Kinetic.
2. Select the **filter(s)** to be used.
3. Define a rectangle of **wells** to be read if reading a partial plate.
4. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
5. For an endpoint **linear scanning** protocol, click the **Scanning…** button to define the horizontal reading points.
6. (Optional) Define **Shaking** parameters.
7. (Optional) Define **Temperature Control** parameters.
8. (Optional) Select **Pre-Read Blank Plate.**
9. (Optional) Select **Read Barcode** to instruct the instrument to scan the barcode as the plate is drawn in to the reading chamber.
10. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
11. Click **OK** to verify the parameters and close this dialog.



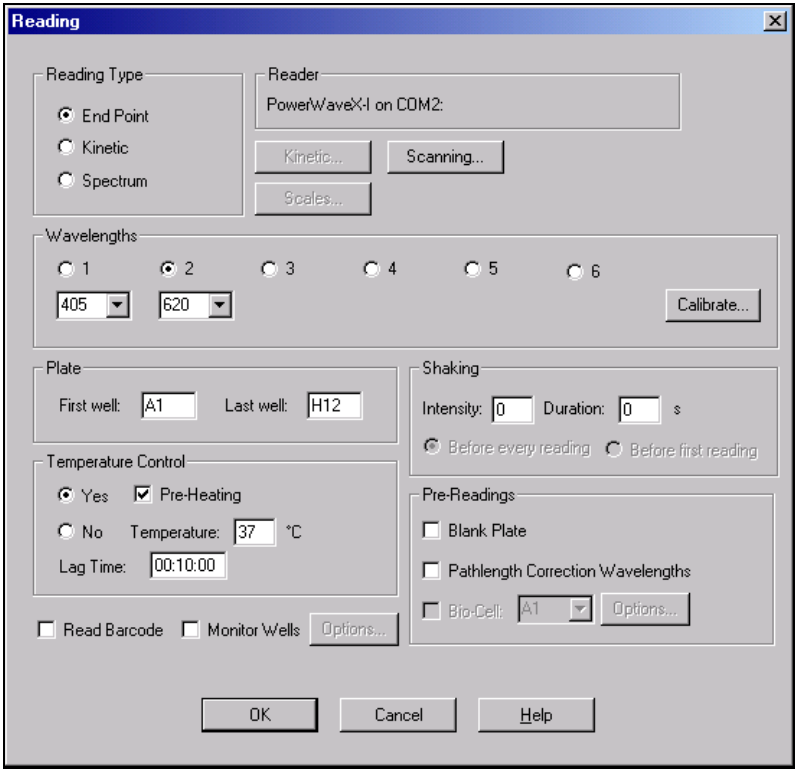
1. Select a **Detection Method** of Absorbance, Fluorescence, or Luminescence.
2. Select a **Reading Type** of Endpoint or Kinetic.
3. For a **fluorescence** or **luminescence** protocol, define the **Filter Sets** to be used.
4. For an **absorbance** protocol, define the **Wavelengths** and **Optics Position** to be used.
5. Select a **Plate Type** and choose a **Reading Direction.**
6. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
7. For an endpoint or kinetic **scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
8. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
9. (Optional) Define **General Options** parameters.
10. (Optional) Define **Shaking** parameters.
11. (Optional) Define **Temperature Control** parameters.
12. (Optional) Select **Pre-Read Blank Plate.**
13. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
14. Click **OK** to verify the parameters and close this dialog.



1. Select a **Detection Method** of Fluorescence or Luminescence.
2. Select a **Reading Type** of Endpoint or Kinetic.
3. (Optional) Click **Update Filters** to retrieve current filter values from the instrument.
4. Define the **Filter Sets** to be used.
5. Select a **Plate Type** and define the **First** and **Last Wells.**
6. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
7. For an endpoint **area scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
8. (Optional) Define **Shaking** parameters.
9. (Optional) Define **Temperature Control** parameters.
10. (Optional) Select **Pre-Read Blank Plate.**
11. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
12. (Optional) Select **Eject Between Filter Set Read.**
13. Click **OK** to verify the parameters and close this dialog.

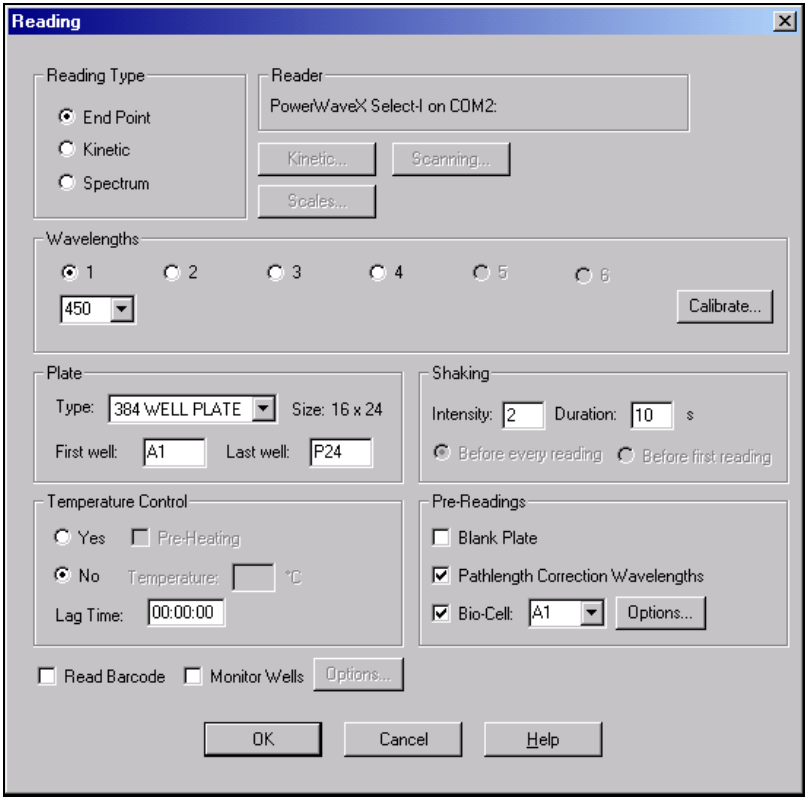


1. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
2. Define the **Wavelengths** and **Read Mode** to be used. Calibrate the wavelengths if necessary.
3. Select a **Plate Type** and define the **First** and **Last Wells.**
4. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
5. For an endpoint **linear scanning** protocol, click the **Scanning…** button to define the horizontal reading points.
6. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
7. (Optional) Define **Shaking** parameters.
8. (Optional) Define **Temperature Control** parameters.
9. (Optional) Select and define **Pre-Reading** options.
10. (Optional) Select **Read Barcode** to instruct the instrument to scan the barcode as the plate is drawn in to the reading chamber.
11. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
12. Click **OK** to verify the parameters and close this dialog.



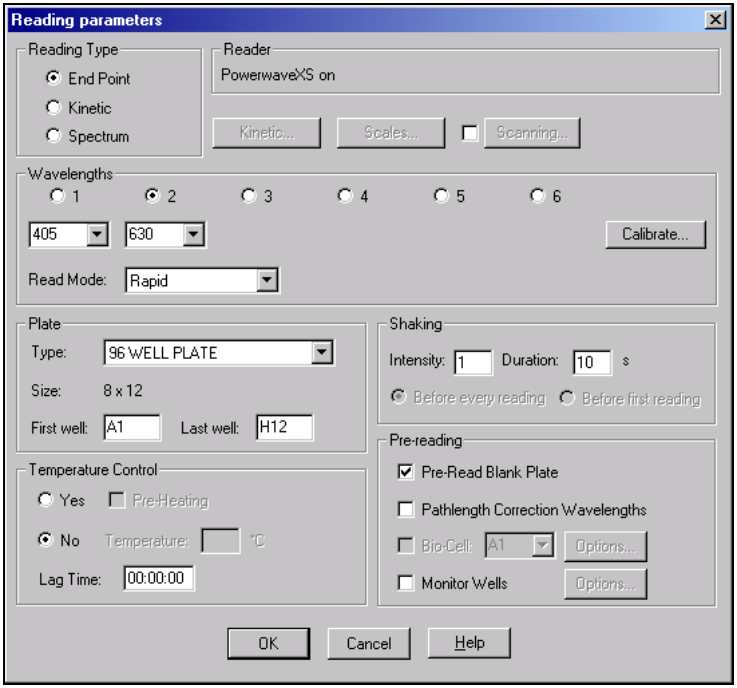
1. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
2. Define the **Wavelengths** to be used. Calibrate the wavelengths if necessary.
3. Define a rectangle of **wells** to be read if reading a partial plate.
4. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
5. For an endpoint **linear scanning** protocol, click the **Scanning…** button to define the horizontal reading points.
6. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
7. (Optional) Define **Shaking** parameters.
8. (Optional) Define **Temperature Control** parameters.
9. (Optional) Select and define **Pre-Reading** options.
10. (Optional) Select **Read Barcode** to instruct the instrument to scan the barcode as the plate is drawn in to the reading chamber.
11. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
12. Click **OK** to verify the parameters and close this dialog.

PowerWaveX™ Select



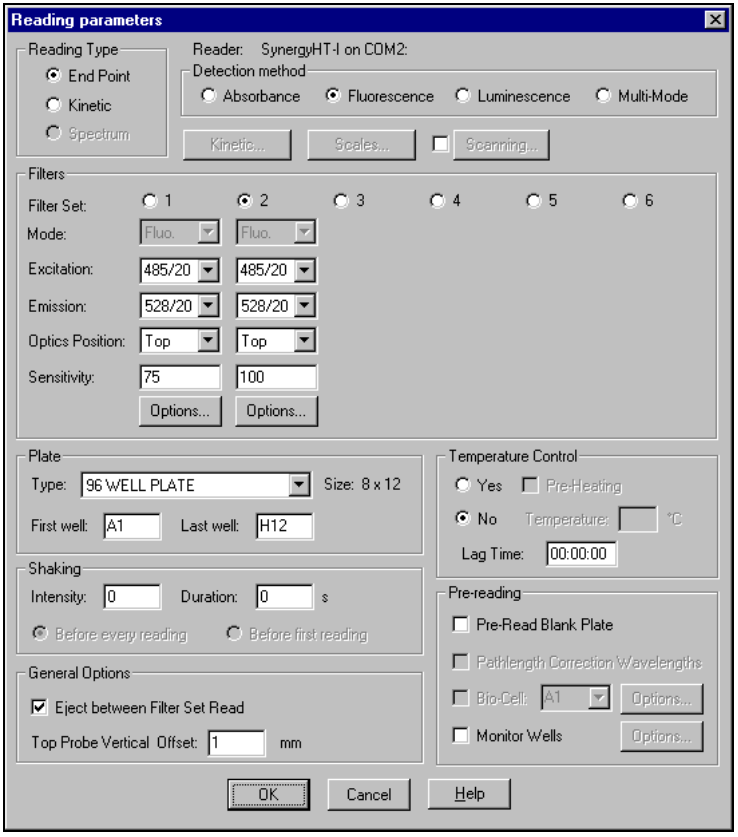
1. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
2. Define the **Wavelengths** to be used. Calibrate the wavelengths if necessary.
3. Select a **Plate Type** and define the **First** and **Last Wells.**
4. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
5. For an endpoint **linear scanning** protocol, click the **Scanning…** button to define the horizontal reading points.
6. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
7. (Optional) Define **Shaking** parameters.
8. (Optional) Define **Temperature Control** parameters.
9. (Optional) Select and define **Pre-Reading** options.
10. (Optional) Select **Read Barcode** to instruct the instrument to scan the barcode as the plate is drawn in to the reading chamber.
11. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
12. Click **OK** to verify the parameters and close this dialog.

PowerWave™ XS



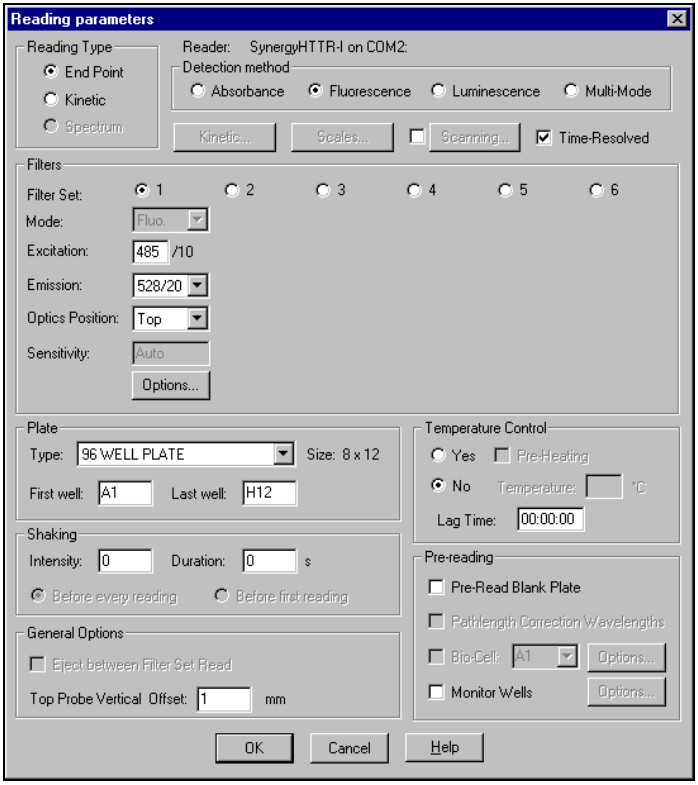
1. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
2. Define the **Wavelengths** and **Read Mode** to be used. Calibrate the wavelengths if necessary.
3. Select a **Plate Type** and define the **First** and **Last Wells.**
4. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
5. For an endpoint **area scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
6. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
7. (Optional) Define **Shaking** parameters.
8. (Optional) Define **Temperature Control** parameters.
9. (Optional) Select and define **Pre-Reading** options.
10. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
11. Click **OK** to verify the parameters and close this dialog.

Synergy™ HT



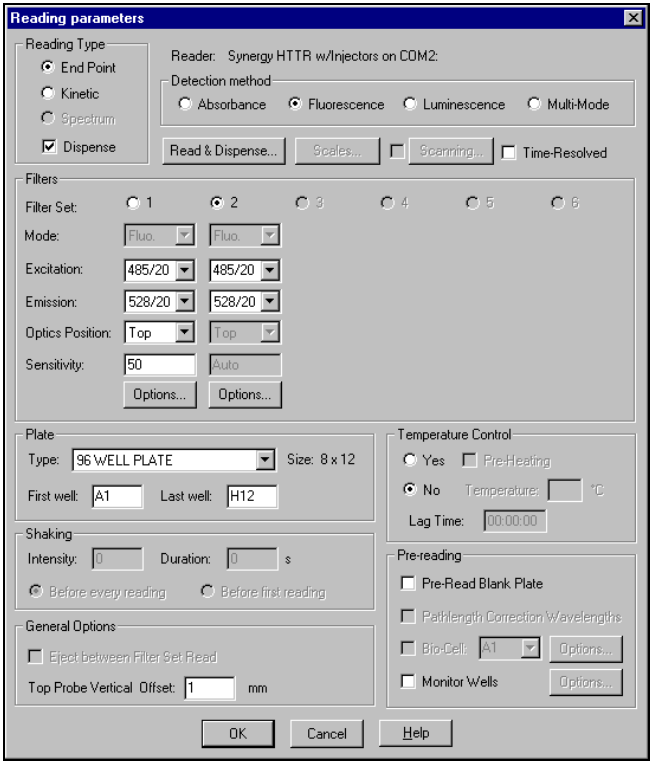
1. Select a **Detection Method** of Absorbance, Fluorescence, Luminescence, or Multi-Mode.
2. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
3. For an **absorbance** protocol, define the **Wavelengths** and **Read Mode** to be used. Calibrate the wavelengths if necessary.
4. For a **fluorescence, luminescence,** or **multi-mode** protocol, define the **Filter Sets** to be used.
5. Select a **Plate Type** and define the **First** and **Last Wells.**
6. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
7. For an endpoint **area scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
8. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
9. (Optional) Define **Shaking** parameters.
10. (Optional) Define **General Options** parameters.
11. (Optional) Define **Temperature Control** parameters.
12. (Optional) Select and define **Pre-Reading** options.
13. Click **OK** to verify the parameters and close this dialog.

Synergy™ HT / Time-Resolved



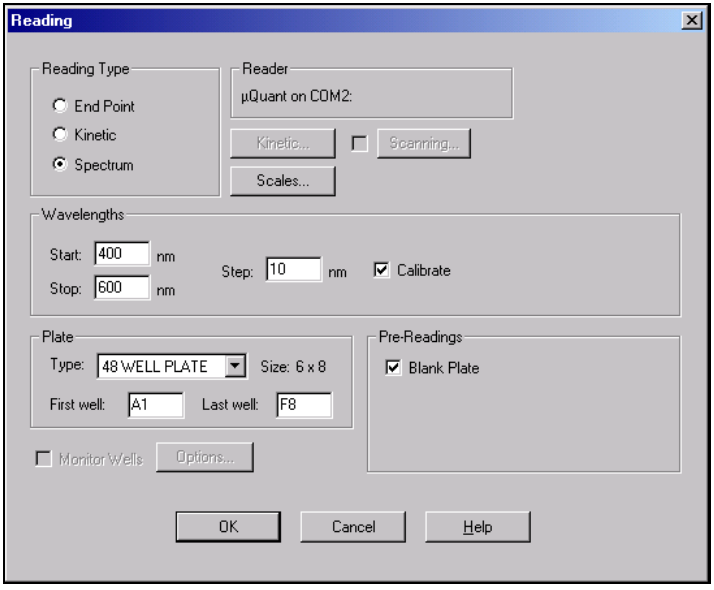
1. Select a **Detection Method** of Absorbance, Fluorescence, Luminescence, or Multi-Mode.
2. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
3. (Optional) For a **fluorescence** protocol, enable the **Time-Resolved** option.
4. For an **absorbance** protocol, define the **Wavelengths** and **Read Mode** to be used. Calibrate the wavelengths if necessary.
5. For a **fluorescence, luminescence,** or **multi-mode** protocol, define the **Filter Sets** to be used.
6. Select a **Plate Type** and define the **First** and **Last Wells.**
7. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
8. For an endpoint **area scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
9. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
10. (Optional) Define **Shaking** parameters.
11. (Optional) Define **General Options** parameters.
12. (Optional) Define **Temperature Control** parameters.
13. (Optional) Select and define **Pre-Reading** options.
14. Click **OK** to verify the parameters and close this dialog.

Synergy™ HT / Time-Resolved / Injectors



1. Select a **Detection Method** of Absorbance, Fluorescence, Luminescence, or Multi-Mode.
2. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
3. (Optional) For a **fluorescence** or **luminescence** protocol, check **Dispense.**
4. (Optional) For a **fluorescence** protocol, enable the **Time-Resolved** option.
5. For a **fluorescence, luminescence,** or **multi-mode** protocol, define the **Filter Sets**.
6. For a **Dispense** protocol, click **Read & Dispense** to define additional parameters.
7. For an **absorbance** protocol, define the **Wavelengths** and **Read Mode** to be used. Calibrate the wavelengths if necessary.
8. Select a **Plate Type** and define the **First** and **Last Wells.**
9. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
10. For an endpoint **area scanning** protocol, enable the Scanning checkbox and then click the **Scanning…** button to define the read matrix size.
11. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
12. (Optional) Define **Shaking** parameters.
13. (Optional) Define **General Options** parameters.
14. (Optional) Define **Temperature Control** parameters.
15. (Optional) Select and define **Pre-Reading** options.
16. Click **OK** to verify the parameters and close this dialog.

µQuant™



1. Select a **Reading Type** of Endpoint, Kinetic, or Spectrum.
2. Define the **Wavelengths** to be used. Calibrate the wavelengths if necessary.
3. Select a **Plate Type** and define the **First** and **Last Wells.**
4. For a **kinetic** protocol, click the **Kinetic…** button to define the read time and interval.
5. For an endpoint **area scanning** protocol, enable the **Scanning** checkbox and then click the **Scanning…** button to define the read matrix size.
6. (Optional) For a **spectrum** protocol, click the **Scales…** button to customize the graphical data display.
7. (Optional) Select and define **Pre-Reading** options.
8. (Optional) Select **Monitor Wells** and then click **Options…** to define related parameters.
9. Click **OK** to verify the parameters and close this dialog.

Reading Parameters Reference

All possible reading parameters and certain related topics are discussed on the following pages. Information is organized alphabetically by parameter name or topic.

Clarity™ Microplate Luminometer users: Refer to Chapter 4 of the **Clarity Operator's Manual** for information on reading parameters.

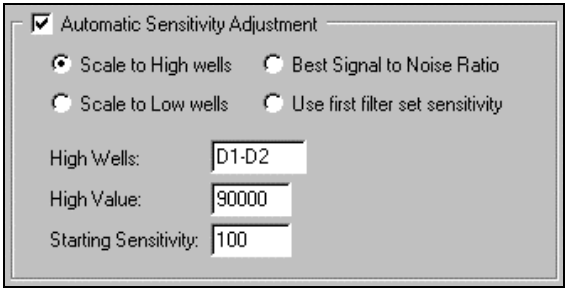
Automatic Sensitivity Adjustment

Enable **Automatic Sensitivity Adjustment** to let KC4 automatically select the most appropriate sensitivity value for a particular filter set. There are three methods to choose from: **Scale to High Wells, Scale to Low Wells,** and **Best Signal to Noise Ratio.** The best method depends on your application (some experimentation may be necessary).

Note for Synergy™ HT w/Injector models. With Dispense protocols, if two filter sets are defined and Automatic Sensitivity Adjustment is enabled for the first filter set, 'Use first filter set sensitivity' is the required selection for the second filter set.

Scale to High Wells

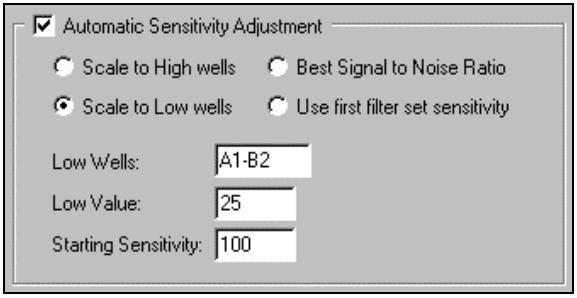
Scale to High Wells searches for two consecutive gain values where the measured values are lower and higher than the defined high well values.



- The **High Wells** represent those wells on which the calibration measurements will be taken. They are typically wells with the highest expected signal (measurement value) on the plate, such as the high standard wells.
- The **High Value** represents the upper limit of the expected range of values for the entire microplate. The default value for the FL600™ is 90000 RFU/RLU with a valid range of 0 to 99999. The default value for the FLx800™ and the Synergy™ HT is 80000 RFU/LFU with a valid range of 0 to 99998. The recommended high value for all of these readers is 50,000 to 70,000 RFU/RLU.
- The default **Starting Sensitivity** is 100. The valid range is from 25 to 255. The recommended starting sensitivity is 25.

Scale to Low Wells

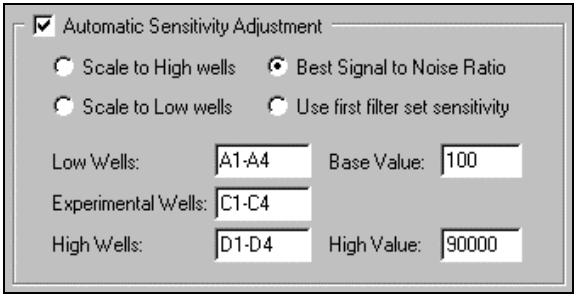
Scale to Low Wells searches for two consecutive gain values where the measured values are lower and higher than the defined low value wells. Scale to Low Wells is recommended for weakly luminescent reactions.



- The **Low Wells** represent those wells on which the calibration measurements will be taken. They are typically wells with the lowest expected signal (measurement value) on the plate, such as blank wells, negative controls, or the zero standard wells.
- The **Low Value** represents the lower limit of the expected range of values for the entire microplate. The default value is 25 RFU/RLU, and the valid range is from 0 to 99999 for the FL600™, 0 to 99998 for the FLx800™ and the Synergy™ HT. The recommended low value for all of these readers is 100 to 200 RFU/RLU.
- The default **Starting Sensitivity** is 100. The valid range is from 25 to 255. The recommended starting sensitivity is 25.

Best Signal to Noise Ratio

With the Best Signal to Noise Ratio method, KC4 instructs the reader to perform several pre-readings on the plate to determine the optimal sensitivity. The optimum sensitivity is based on the best signal-to-noise ratio obtained during these pre-readings.



- Designate certain wells on the plate as Low, Experimental, and High Wells. Enter a single well location (ex. A1) or a 'rectangle' (ex. B1-C2) of well locations.

  Low Wells are usually blank wells, buffer wells, or negative controls, and are expected to yield a low RFU or RLU value.

  Experimental Wells are usually low standards or low positive controls, and are expected to yield a mid-range RFU or RLU value.

  High Wells are usually the highest standards or high positive controls, and are expected to yield a high (but not over range) RFU or RLU value.

- Specify the Base and High Values.

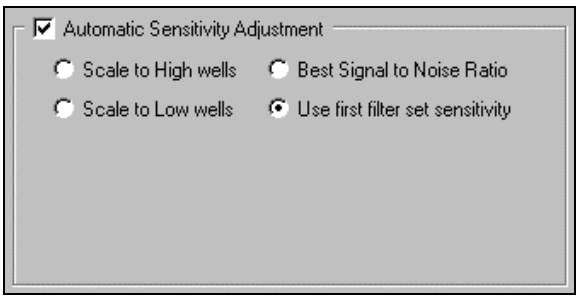
  The **Base Value** is the fluorescence or luminescence value that the Low and Experimental Wells must exceed during sensitivity determination. This ensures that the plate produces values that are large enough to be meaningful.

  The **High Value** is the fluorescence or luminescence value that the High Well(s) should approach but not exceed. The default setting is 90000 RFU/RLU, and the maximum value is 99999 for the FL600™ and 99998 for the FLx800™ and the Synergy™ HT. Practically, this value could be anywhere from 10000 to 90000.

  Note: If the Best signal to Noise Ratio cannot be calculated, use Scale to High Wells.

Use First Filter Set Sensitivity

If multiple filter sets are defined, you can opt to use the sensitivity determined with filter set #1 for other filter sets, by choosing Use First Filter Set Sensitivity.


Automatic Sensitivity Adjustment
Scale to High wells
Best Signal to Noise Ratio
Scale to Low wells
Use first filter set sensitivity

Bandwidth Verification

(Only applies to fluorescence/luminescence readers.)

KC4 evaluates each filter set against two criteria:

1) Overlap Evaluation

By definition, the bandwidth rating on the filters used for standard fluorescence reads represents the bandwidth at the 50% transmission level. The transmission profile of these filters is essentially rectangular and therefore the bandwidth does not vary very much when the transmission levels change. When KC4 checks for overlap it ensures that the excitation and emission filter center wavelengths are far enough apart that there is no overlap of their bandwidths. The formula used to evaluate each pair of filter sets for overlap is as follows:

|CW excitation - CW emission| > (BW excitation + BW emission)/2, where:

CW excitation is the center wavelength of the excitation filter
CW emission is the center wavelength of the emission filter
BW excitation is the bandwidth of the excitation filter*
BW emission is the bandwidth of the emission filter

* If the Time-Resolved option is enabled, the value of BW excitation must be multiplied by 2 prior to substituting it into the formula to account for the triangular shape of the transmission profile when using the monochromator. Specifically, although the monochromator has a bandwidth of 10 nm at 50% transmission levels, it approximately doubles by the time the transmission levels approach zero.

If the absolute value of the difference in the excitation and emission center wavelengths is greater than half the sum of the excitation and emission bandwidths, then no overlap condition exists. If it is less than or equal to, then an overlap condition does exist.

- When all of the reading parameters have been defined and the user attempts to validate them (by clicking OK), to KC4 evaluates each filter set pair against the overlap formula.
  - If the result is such that there is no overlap condition for any of the defined filter set pairs, and there are no other parameter definition violations, KC4 will allow you to "OK" out of the Reading Parameters dialog.
  - If the result is such that there is an overlap condition for any filter set pair, KC4 will display a message resembling the following: "Please select alternative filters for filter set #1. The bandpasses overlap." KC4 will not allow you to "OK" out of the Reading Parameters dialog until there are no overlapping filter set pairs.

2) Proximity Evaluation

Although the transmission profile is quite vertical, it does "flair out" at very low transmission levels indicating an increase in the bandwidth. In order to account for the bandwidth at these lower transmission levels, KC4 performs a proximity check on each filter set pair. The formula used to evaluate each pair of filter sets to determine if they are "too close" to each other is as follows:

|CW excitation - CW emission| > BW maximum + (BW minimum/2), where:

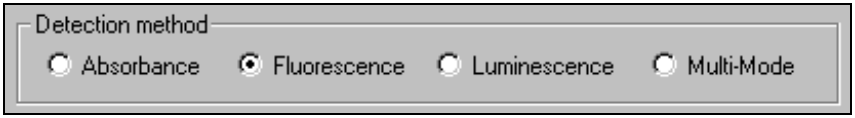
CW excitation is the center wavelength of the excitation filter
CW emission is the center wavelength of the emission filter
BW maximum is the larger of the two bandwidths*
BW minimum is the smaller of the two bandwidths*

* If the Time-Resolved option is enabled, the determination of BW maximum and BW minimum must be made by comparing the emission bandwidth to twice the excitation bandwidth. Once again, this accounts for the triangular shape of the transmission profile when using the monochromator.

If the absolute value of the difference in the excitation and emission center wavelengths is greater than the sum of the maximum bandwidth and half the minimum bandwidth, then no proximity condition exists. If it is less than or equal to, then a proximity condition does exist.

- When all of the reading parameters have been defined and the user attempts to validate them (by clicking OK), to KC4 evaluates each filter set pair against the proximity formula.
  - If the result is such that there is no proximity condition for any of the defined filter set pairs, and there are no other parameter definition violations, KC4 will allow you to "OK" out of the Reading Parameters dialog.
  - If the result is such that there is proximity condition for any filter set pair, KC4 will display a message resembling the following: "Warning: the bandpasses for filter set #2 are too close." However, KC4 does not prohibit you from saving and running protocols that produce proximity warning messages.

Detection Method



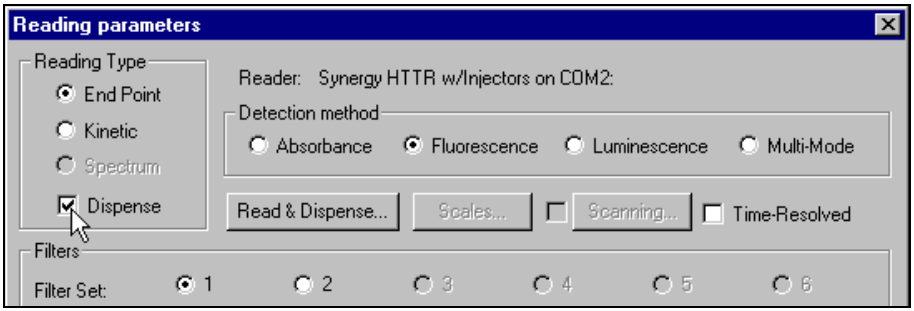
Certain readers, such as the FL600™, FLx800™, and Synergy™ HT, have multi-detection read capability. Before defining additional Reading parameters, click the radio button associated with the detection method required for the assay.

- **Multi-Mode** is only available for the Synergy HT line of instruments. When Multi-Mode is selected, more than one detection method can be defined within a single protocol.

When a Detection Method is selected, KC4 customizes the Reading Parameters dialog so that only the options supported by the Detection Method are available for selection.

The Detection Method selection also instructs KC4 to display measurement values as OD (optical density), RFU (relative fluorescence unit), or RLU (relative luminescence unit).

Dispense Option



The Synergy™ HT with Injectors provides the ability to dispense fluid into microwells as part of a **Read & Dispense** protocol.

When the Dispense option is enabled, the following limitations are applied:

- Area scanning is not supported.
- 1 or 2 filter sets may be defined.
- The Shaking options are disabled within the main Reading Parameters dialog. A Shake step may be added to Plate Mode readings in the Read & Dispense dialog.
- The Lag Time option is disabled.
- The Eject Between Filter Sets option is disabled.
- The Top Probe Vertical Offset option is disabled. The top probe height will be automatically set to 1 mm above the top of the plate.

Note: The Dispense option is not available when Multi-Mode is selected.

Eject Between Filter Set Read

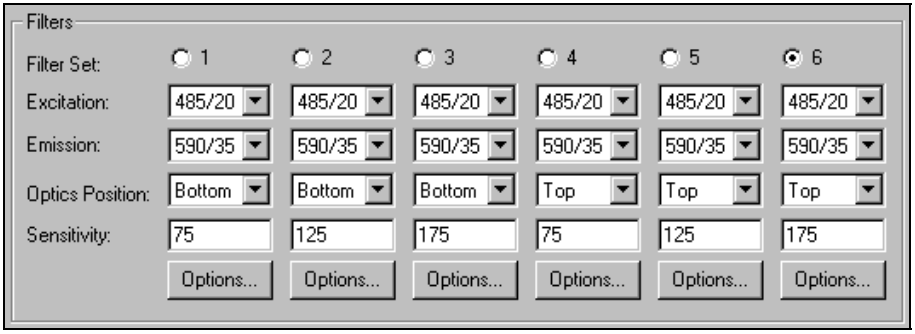
This feature is available for fluorescence and luminescence protocols if you are defining an endpoint read with two or more filter sets. Enable **Eject between Filter Set Read** to:

- Perform an initial reading of a blank plate, followed by the assay plate(s) at some later time.
- Provide the ability to add reagents to the same plate between readings.
- Enable reading up to 6 different plates with one protocol and plate file.

If this feature is enabled, KC4 ejects the plate after one filter set read, then displays a message to click OK to start the next filter set read.

See also "General Options (FL600)" or "General Options (Synergy HT)."

Excitation/Emission Filter Sets (FL600™)



The FL600 can take measurements with up to six uniquely defined Filter Sets. There are many applications for **multiple filter sets,** including:

- Assays that have more than one fluorophore in the well.
- Comparison of results between the top and bottom optics positions.
- Comparison of results between rapid and static mode.
- Comparison of results with varying static mode settings.
- Comparison of results with varying sensitivity settings.

To define **Filter Sets:**

- Ensure the protocol is appropriately set for **Absorbance, Fluorescence,** or **Luminescence** detection method.
- Select the **Filter Set** radio button corresponding with the number of sets required.
- Define the wavelengths:
  - **Absorbance detection method:**

    For each filter set, use the drop-down lists to select the desired wavelength.
  - **Fluorescence detection method:**

    For each filter set, use the drop-down lists to select the **Excitation** and **Emission** wavelengths.

- **Luminescence detection method:**

  For maximum sensitivity, select **Lum/E** to indicate an empty position in the filter wheel and a plug in position E in the excitation wheel.

  For each filter set, use the drop-down lists to select the **Emission** wavelength.

- **Note:** To prevent damage to the reader, KC4 performs bandwidth verification as part of the validation within the Reading Parameters dialog.
- The instrument may be equipped with one or two optical probes, positioned above (Top) and/or below (Bottom) the assay plate. Specify the **Optics Position** to be used for each filter set.

  Tips:

  - Evaluate the proper reading position by specifying two (or more) filter sets, one each for the Top and Bottom optics positions.
  - The top position is advantageous for homogeneous solutions using solid black plates.
  - The bottom position is advantageous for cellular assays and assays requiring a covered plate to maintain sterility.
  - The recommended Optics Position for Absorbance readings is Bottom. KC4 provides a warning if Top is selected, although this selection will not be prohibited.
  - The recommended Optics Position for Luminescence readings is Top.

- Fluorescence or Luminescence method only: Specify the **Sensitivity** setting for each individual filter set. Valid settings are **0** and integers from **25** to **255. 'Auto'** indicates that the Auto Sensitivity Adjustment feature has been enabled.

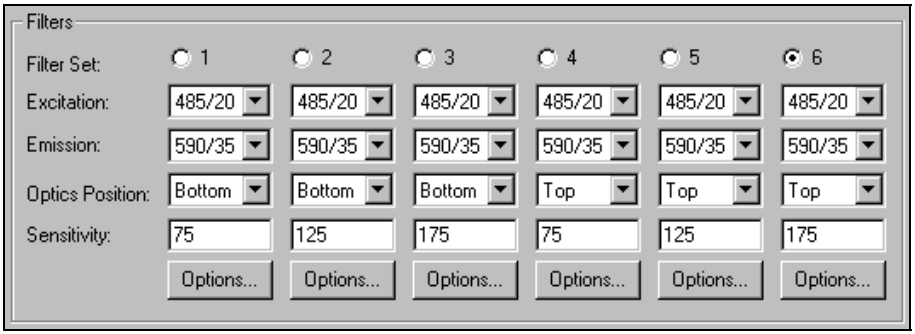
  The signal from the range of fluorescence and luminescence assays can be very weak, very strong, or anywhere in between. For each assay, the **sensitivity** of the photomultiplier (PMT) should be adjusted to ensure that the signals from all wells fall within the appropriate dynamic range of 0 to 99999 relative fluorescence or luminescence units (RFU or RLU).

  Tips:

  - Typical assays using 96- (or fewer) well plates require sensitivities between 75 and 190. Assays using 384-well or other higher-density plates will likely require much higher sensitivities due to narrower optical probes. Luminescent reactions may require sensitivities up to 255.
  - To determine the optimum sensitivity for an assay, use Scale to High Wells or Scale to Low Wells. Scale to High Wells is used for fluorescence and bright luminescence assays. Scale to Low Wells is used for weakly luminescent reactions. See Automatic Sensitivity Adjustment for more information.
  - As the sensitivity setting increases, so will the fluorescence values. If many wells result in "OVRFLW" (indicating RFU or RLU values greater than 99999), the sensitivity setting is probably too high.
  - If each well contains more than one fluorophore, one of which might give brighter or weaker results, assign each filter set its own sensitivity.
  - If the field shows **'Auto',** the Automatic Sensitivity Adjustment feature is enabled.

- Click the **Options…** button to specify the Measurement Options for each *individual* filter set (Endpoint Readings), or once for all filter sets (Kinetic Readings).

Excitation/Emission Filter Sets (FLx800, Synergy HT)



The FLx800™ and Synergy™ HT can take measurements with up to six uniquely defined Filter Sets for endpoint reads and up to two for kinetic reads. There are several applications for **multiple filter sets,** including:

- Assays that have more than one fluorophore in the well.
- Comparison of results between the top and bottom optics positions.
- Comparison of results with varying sensitivity settings.

To define **Filter Sets:**

- Select either the **Fluorescence** or **Luminescence** detection method.
- Select **Endpoint** or **Kinetic** reading type.
- Click the **radio button** corresponding with the number of Filter Sets required.

  Endpoint reads without area scanning: 1 to 6

  Endpoint reads with area scanning: 1 or 2

  Kinetic reads: 1 or 2

  Dispense feature enabled: 1 or 2
- Define the wavelengths:
  - **Fluorescence detection method:**

    For each filter set, use the drop-down lists to select the **Excitation** and **Emission** wavelengths.
  - **Fluorescence detection method with Time-Resolved enabled (Synergy HTTR):**

    For each **Excitation** filter set, enter a wavelength value between 200 and 999 nm. The bandpass is not variable; it is pre-defined to be 10 nm.

    For each **Emission** filter set, use the drop-down lists to select the wavelengths.
  - **Luminescence detection method:**

    For maximum sensitivity, select **Lum/E** to indicate an empty position in the filter wheel and a plug in position E in the excitation wheel.

    For each filter set, use the drop-down lists to select the **Emission** wavelength.
- **Note:** To prevent damage to the reader, KC4 performs bandwidth verification as part of the validation within the Reading Parameters dialog.

- The instrument may be equipped with one or two optical probes, positioned above (Top) and/or below (Bottom) the assay plate. Specify the **Optics Position** to be used for each filter set.

  Tips:

  - Evaluate the proper reading position by specifying two (or more) filter sets, one each for the Top and Bottom optics positions.
  - The top position is advantageous for homogeneous solutions using solid black plates.
  - The bottom position is advantageous for cellular assays and assays requiring a covered plate to maintain sterility.
  - The recommended Optics Position for Luminescence readings is Top.

- Specify the **Sensitivity** setting for each individual filter set. Valid settings are **0** and integers from **25** to **255. 'Auto'** indicates that the Auto Sensitivity Adjustment feature has been enabled.

  Note: With **Dispense** protocols, if two filter sets are defined the field for the second filter set always shows **Auto** and will use the first filter set's sensitivity.

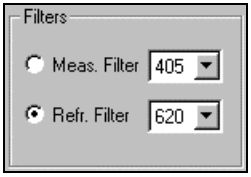
  The signal from the range of fluorescence and luminescence assays can be very weak, very strong, or anywhere in between. For each assay, the sensitivity of the photomultiplier (PMT) should be adjusted to ensure that the signals from all wells fall within the appropriate dynamic range of 0 to 99998 relative fluorescence or luminescence units (RFU or RLU).

  Tips:

  - Typical assays using 96- (or fewer) well plates require sensitivities between 35 and 150. Assays using 384-well or other higher-density plates will likely require much higher sensitivities due to narrower optical probes. Luminescent reactions may require sensitivities up to 255.
  - To determine the optimum sensitivity for an assay, use Scale to High Wells or Scale to Low Wells. Scale to High Wells is used for fluorescence and bright luminescence assays. Scale to Low Wells is used for weakly luminescent reactions. See Automatic Sensitivity Adjustment for more information.
  - As the sensitivity setting increases, so will the fluorescence values. If many wells result in "OVRFLW" (indicating RFU or RLU values greater than 99998), the sensitivity setting is probably too high.
  - If each well contains more than one fluorophore, one of which might give brighter or weaker results, assign each filter set its own sensitivity.
  - If the field shows **'Auto',** the Automatic Sensitivity Adjustment feature is enabled.

- Click the **Options…** button to specify the Measurement Options for each *individual* filter set.

Filters



The **Filters** parameters include:

- **Meas. Filter:** select the measurement filter for a single- or dual- wavelength read.
- **Refr. Filter:** select the reference filter for a dual-wavelength read.

First Well / Last Well

Most readers support reading a subset of the entire plate, based on contiguous microplate wells.

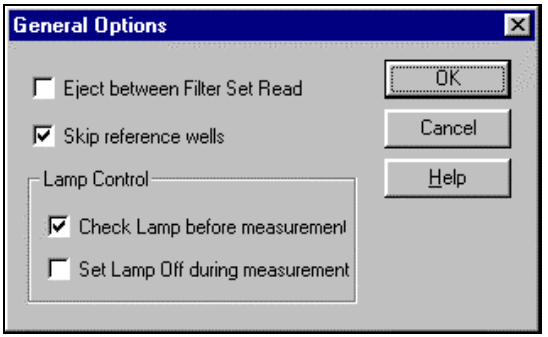
To specify the range of wells to be read:

- If applicable, select the appropriate **Plate Type.** The default **Last Well** field value will automatically update to match the selected plate type.
- Set the **First Well** and **Last Well** fields to indicate the range of wells to be read. For example, if First Well is set to **A1** and Last Well is set to **B3,** KC4 reads a 'rectangle' of wells: A1, A2, A3, B1, B2, and B3.

General Options (FL600™)

KC4 provides options for controlling certain reader functions preceding and during plate reads, such as lamp control and plate ejection.

Click the **Options** button from within the Reading Parameters dialog to open the **General Options** dialog. The available/recommended settings vary, depending on the reading mode.



Absorbance mode:

- Enable **Eject between Filter Set Read** to perform an initial reading of a blank plate followed by the assay plate(s) at some later time; provide the ability to add reagents to the same plate between readings; or enable reading up to 6 different plates with one protocol and plate file.

  This feature is available for Endpoint reads only.

- **Skip reference wells** is selected for absorbance readings, and cannot be changed.
- Enable **Check Lamp before measurement** to check lamp output prior to measurement. This feature is selected by default. Deselecting this feature speeds up the reading process, however, a lamp output failure may not be detected.
- Enable **Set Lamp Off during measurement** to allow for the reading of an unexcited signal. This feature is deselected by default.

Fluorescence mode:

- Enable **Eject between Filter Set Read** to perform an initial reading of a blank plate followed by the assay plate(s) at some later time; provide the ability to add reagents to the same plate between readings; or enable reading up to 6 different plates with one protocol and plate file.

  This feature is available for Endpoint reads only.

- **Skip reference wells** is deselected by default.

  The FL600 is equipped with fluorescent plastic compounds call **reference wells** which help provide the best results when using Rapid Mode to read the assay plate. Skip reference wells should be deselected for use with Rapid Mode reads**.**

- Enable **Check Lamp before measurement** to check lamp output prior to measurement. This feature is selected by default. Deselecting this feature speeds up the reading process, however, a lamp output failure may not be detected.
- Enable **Set Lamp Off during measurement** to allow for the reading of an unexcited signal. This feature is deselected by default.

Luminescence mode:

- Enable **Eject between Filter Set Read** to perform an initial reading of a blank plate, followed by the assay plate(s) at some later time; provide the ability to add reagents to the same plate between readings; or enable reading up to 6 different plates with one protocol and plate file.

  This feature is available for Endpoint reads only.

- **Skip reference wells** is deselected for luminescence readings, and cannot be changed.
- **Check Lamp before measurement** is deselected by default. The lamp does not need to be checked before measurement.
- **Set Lamp Off during measurement** is selected for luminescence readings, and cannot be changed. The lamp is turned off during measurement to avoid damage to the photomultiplier.

Reading the Reference Wells

It is strongly recommended that you read the reference wells if **Rapid Mode** is enabled. If, however, reading the Reference Wells **must** be skipped, consider the following:

- During an **Endpoint** read, when prompted to "Insert plate and press OK", do so within 60 seconds.
- If a **Lag Time** is specified for an Endpoint or Kinetic read, the maximum recommended time is 60 seconds. If a longer lag time is required, read the reference wells first or use Static Mode to read the plate.
- During a **Kinetic** read, the maximum time interval is 60 seconds. If a longer time interval is required, read the reference wells first or use Static Mode to read the plate.

If the Reference Wells are skipped and the above time limits are exceeded, the FL600 can provide unexpectedly high fluorescence values in the first well that is read on the assay plate. Usually the first well is A1 but since the software provides the ability to change the starting well, the first well can be anywhere on the plate.

General Options (Synergy™ HT)

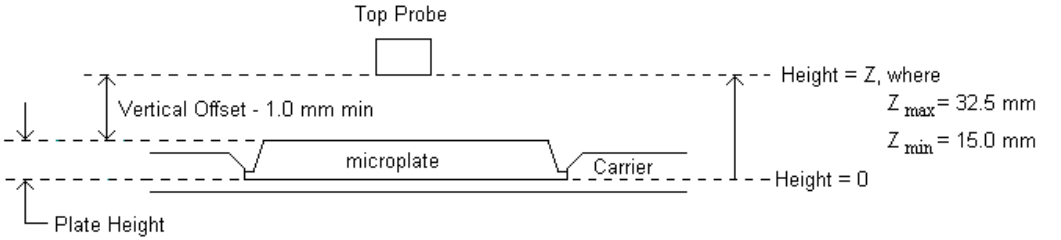
KC4 provides options for controlling certain reader functions preceding and during plate reads, such plate ejection and top probe positioning.

If you are defining an endpoint read with two or more filter sets and are utilizing either the fluorescence or luminescence detection method, enable **Eject between Filter Set Read** to:

- Perform an initial reading of a blank plate, followed by the assay plate(s) at some later time.
- Provide the ability to add reagents to the same plate between readings.
- Enable reading up to 6 different plates with one protocol and plate file.

  If this feature is enabled, KC4 ejects the plate after one filter set read, then displays a message to click OK to start the next filter set read.

The **Top Probe Vertical Offset** parameter is available for fluorescence or luminescence reads when at least one of the filter sets uses the "top" optics position. It allows you to define how far the top probe is positioned from the top surface of the plate during the read. The valid range for the offset parameter is determined on a plate by plate basis. The calculation is based on the plate height and the maximum travel of the top probe:



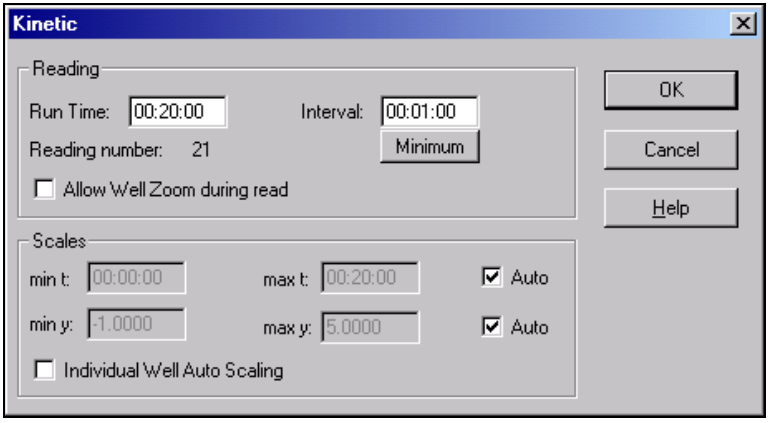
In order to be considered valid, the vertical offset value must satisfy the following conditions:

- 1.0 mm <= offset <= 32.5 - plate height*
- offset >= 15.0 mm - plate height*

* Plate heights are defined via System|Plate Formats.

Kinetic Parameters

If the Kinetic reading type is selected in the Reading dialog, click the **Kinetic…** button to define related parameters.



Notes:

- If the **FL600™** is the current reader, the Kinetic dialog contains some additional parameters. See the next section for more information.
- The default **time format** for kinetics is HH:MM:SS. To change the time format, select System|Setup.

Specify the total kinetic Run Time and the Kinetic Interval. KC4 automatically generates the Reading Number based on these settings.

- **Run Time:** Defines the duration of the complete kinetic run. The maximum run time is 48 hours.

- **Interval:** Defines the time interval between individual kinetic reads. The maximum valid Interval is equal to the current Run Time. The minimum valid Interval depends on the current reader type and certain protocol parameters, such as the number of wavelengths specified, the plate format, the number of wells to be read, and whether or not shaking is selected.

  For certain readers, a **Minimum** button is available under the Interval box. When this button is clicked, KC4 communicates with the reader to determine the minimum kinetic interval based on the currently selected Reading parameters.

  For all readers, when the **OK** button is clicked, KC4 automatically checks the validity of the Interval value. If it is insufficient for the currently defined Reading parameters, a theoretical minimum value is displayed.

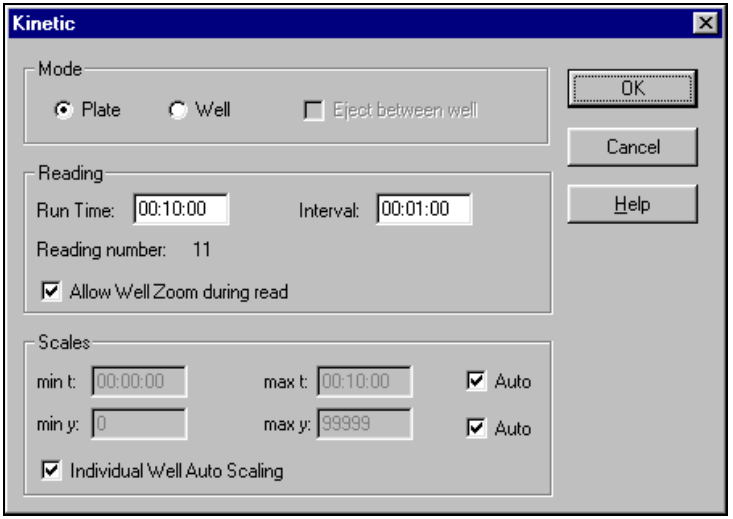
  Note: For readers that support more than one read mode (speed), the returned value is based on the current reading mode. For some instruments, the read mode is set through the instrument's front panel. For others, it is defined as part of the protocol.

- **Reading Number:** Represents the total number of kinetic reads. KC4 calculates this automatically, based on the currently defined Run Time and Interval. The maximum number of kinetic reads is 300.

- **Allow Well Zoom During Read:** If this feature is enabled, you can click on one or more wells in the Results dialog to view the kinetic curve(s) as the plate is being read. KC4 updates the graph and kinetic calculations as each measurement value is retrieved from the reader. **Important!** When the Allow Well Zoom During Read box is checked, a warning message appears. If the computer speed is less than 100 MHz, or if there is insufficient memory, this feature should not be used.

Kinetic Parameters (FL600)

The **Kinetic** dialog contains special **Mode** parameters for the **FL600™.**

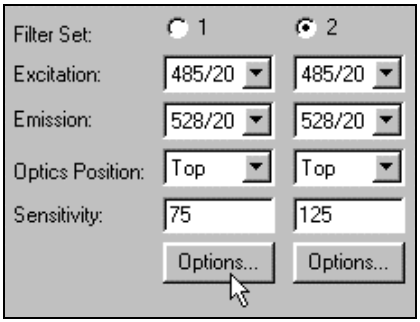


- In **Plate Mode,** the FL600 repeatedly reads a whole or partial plate for a specified number of times and for up to 6 filter sets.

- In **Well Mode,** the FL600™ repeatedly reads a well for a specified number of times for up to 2 filter sets. This mode is typically used for fast kinetic reactions.

  Check **Eject between well** to eject the assay plate between well readings. This feature is useful, for example, for accommodating reagent addition.

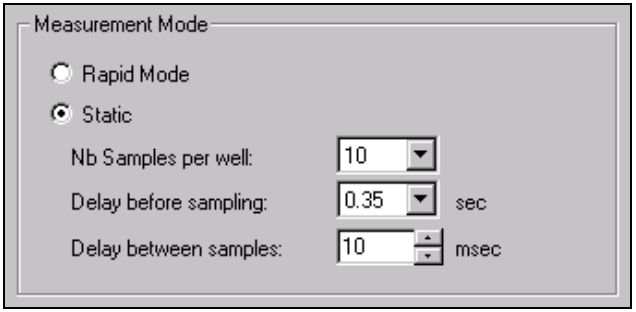
Measurement Options



To define **Measurement Options** such as Sampling or Automatic Sensitivity Adjustment for a particular filter set, start by clicking its **Options** button.

Note: If the current reader is the FL600 and the reading type is Kinetic, click the Options button associated with Filter Set 1. Options selected here are applied to all defined filter sets.

Measurement Mode (FL600)



To define the measurement mode parameters for the FL600:

- Choose between **Rapid** and **Static** mode:
  - **Rapid Mode** reads each well while the assay plate is in motion. This provides for faster reading than with Static mode, but the CVs might be lower.

    The FL600 is equipped with fluorescent plastic compounds called **reference wells** which help provide the best results when using Rapid Mode to read the assay plate. See "General Options (FL600)" for more information.
  - **Static Mode** performs a specified number of reads in each well, with a specified delay between each read. The reading performs more slowly than with Rapid Mode, but the CVs are usually better. **Nb Samples, Delay before sampling,** and **Delay between samples** pertain specifically to Static Mode.

If Static Mode was selected, specify the **Sampling** options:

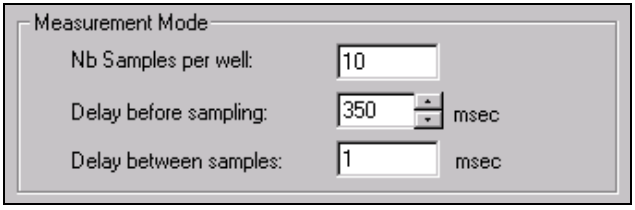
- **Nb Samples** is the number of measurements taken per well. Valid options are 1, 2, 3, 4, 10, 20, 50, and 100 measurements. The value reported for each well represents the *average* of its measurements.

  Usually, the more samples per well the better the CVs, although selecting a large number of samples per well typically results in only marginal improvement. Consider a setting that represents the optimal combination of sensitivity and speed. The recommended setting to achieve a balance between speed and sensitivity is 10 measurements per well. **Note:** The reader takes approximately 10 milliseconds to perform each measurement.

- **Delay before sampling** is the pause time of the optical probe above or below the microwell before the measurement is taken. Valid options are 0.10, 0.35, 0.50, and 2.00 seconds. The recommended setting for fluorescence assays to ensure the probe is stopped for measurement, but for the minimum amount of time is 0.35 seconds. For luminescence reads that allow long reading times, a delay of 2.0 seconds will provide lower CVs. This delay of 2.0 seconds is not recommended for fluorescence reads.

- **Delay between samples** is the time delay between measurements taken in each well. The valid range is from 0 to 2550 milliseconds. The recommended setting is 10 milliseconds. Longer delays between samples may result in better CVs between replicates.

Measurement Mode (FLx800, Synergy HT)



To define the measurement mode parameters for the FLx800™ and Synergy™ HT:

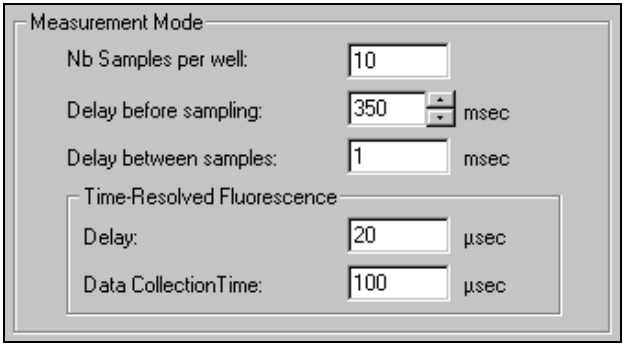
- **Nb Samples** is the number of measurements taken per well. The valid range is from 1 to 255 measurements. The value reported for each well represents the *average* of its measurements.

  Usually, the more samples per well the better the CVs, although selecting a large number of samples per well typically results in only marginal improvement. Consider a setting that represents the optimal combination of sensitivity and speed. The recommended setting to achieve a balance between speed and sensitivity is 10 measurements per well. **Note:** The reader takes approximately 10 milliseconds to perform each measurement.

- **Delay before sampling** is the pause time of the optical probe above or below the microwell before the measurement is taken. The valid range is from 10 to 2550 milliseconds. The recommended setting to ensure the probe is stopped for measurement, but for the minimum amount of time, is 350 milliseconds.

- **Delay between samples** is the time delay between measurements taken in each well. The valid range is from 0 to 255 milliseconds. The recommended setting is 1 millisecond. Longer delays between samples may result in better CVs between replicates.

Measurement Mode (Synergy™ HT / Time-Resolved)



Specify the **Sampling** options:

- **Nb Samples** is the number of measurements taken per well. The valid range is from 1 to 255 measurements. The value reported for each well represents the average of its measurements.

  Usually, the more samples per well the better the CVs, although selecting a large number of samples per well typically results in only marginal improvement. Consider a setting that represents the optimal combination of sensitivity and speed. The recommended setting to achieve a balance between speed and sensitivity is 10 measurements per well.

- **Delay before sampling** is the pause time of the optical probe above or below the microwell before the first flash. The valid range is from 10 to 2550 milliseconds. The recommended setting to ensure the probe is stopped for measurement, but for the minimum amount of time is 350 milliseconds.

- **Delay between samples** is the time delay between the end of data collection and the beginning of the next flash in each well. The valid range is from 0 to 255 milliseconds. The recommended setting is 5 milliseconds. Longer delays between samples may result in better CVs between replicates.

- **Note for Injector models.** The following values are "fixed" for Dispense protocols:

Plate Mode:	Well Mode:
Nb Samples per well: 10	Nb Samples per well: 1
Delay before sampling: 350	Delay before sampling: 10
Delay between samples: 24	Delay between samples: 0

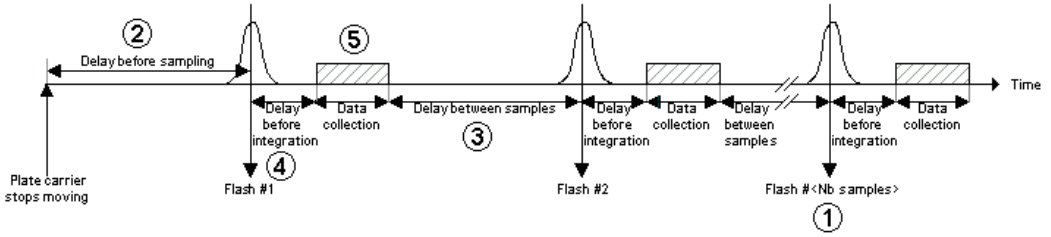
If the **Time-Resolved** option in the Reading Parameters dialog is checked, specify the Time-Resolved Fluorescence options:

- **Delay** is time delay between the flash and the beginning of data collection. It is also referred to as the delay before integration. Valid values are 0 µsec and 20 to 16000 µsec. The default value is 20 µsec.

  Note: A delay of 0 µsec is not the same as a fluorescence read with the time-resolved option turned off. They differ in two ways: (1) the excitation wavelength is generated by the monochromator; and (2) the user can specify the data collection time which is not an available parameter for normal fluorescence reads.

- **Data Collection Time** is the amount of time for which readings are collected after the delay before integration time has expired. The valid range is from 20 to 16000 µsec. The default value is 100 µsec.

The following is a simplified schematic of the measurement mode parameters:



1 = Nb Samples per well

2 = Delay before sampling

3 = Delay between samples

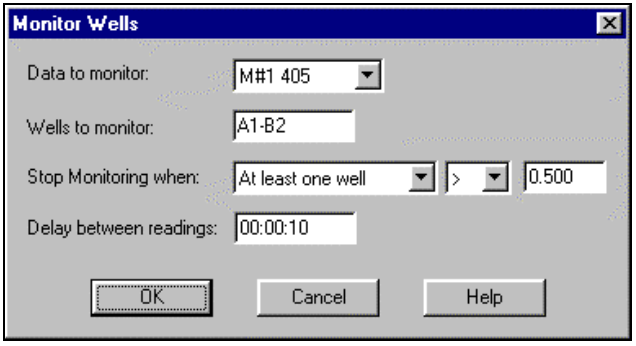
4 = Delay

5 = Data Collection Time

Monitor Wells

If the currently selected reader supports well monitoring, the **Monitor Wells** option is available. The feature enables the instrument to repeatedly read a single well or a group of wells and evaluate the measurements against pre-defined criteria. Once the criteria are met, the plate read begins.

Click the **Options** button to define the criteria:

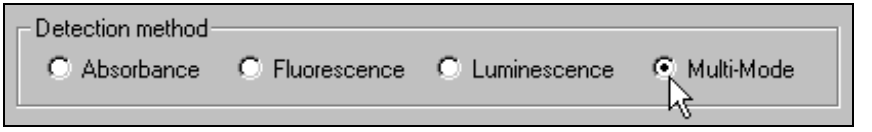


- Select the **Data to Monitor** representing the filter or wavelength value to be used during well monitoring. The available options depend on the current selections in the Reading dialog. Only measured raw absorbance, fluorescence, or luminescence values are presented for selection.

- Enter the well or **Wells to Monitor.** Multiple wells must be contiguous and entered as a range, e.g. A1-A2, or G10-H12.
- Define the **Stop Monitoring When** criteria, to tell the instrument when to conclude the monitoring process:
  - Choose a **conditional option:** 'At least one well' in the range must meet the criteria; the 'Average' of the monitored wells must meet the criteria; or 'All wells' in the range must meet the criteria.
  - Choose an **operator:** >, >=, <, or <=.
  - Enter the **measurement value** to be met. For Absorbance protocols, the valid range is -1.000 to 5.000. For Fluorescence and Luminescence protocols, the valid range is 0 to 99998 (FLx800™ and Synergy™ HT) or 99999 (FL600™).
- If desired, enter a **Delay Between Readings** of the monitored well(s), in HH:MM:SS format. The default setting of 00:00:00 allows the well(s) to be read as quickly as possible.

Multi-Mode

(Only applies to the Synergy™ HT line of instruments.)



Synergy HT instruments with basecode versions of 2.10 or higher support KC4's **Multi-Mode** detection method option. When Multi-Mode is selected, more than one detection method can be defined within a single protocol.

When Multi-Mode is enabled, the following Reading parameters are supported:

- End Point reading type with up to 6 wavelengths or filter sets
- Kinetic reading type with up to 6 wavelengths or filter sets
- Time-Resolved Fluorescence (HTTR)
- Pre-Read Blank Plate
- Monitor Wells
- Automatic Sensitivity Adjustment

The following Reading parameters are **not** supported:

- Rapid and Sweep read modes (absorbance reads are always performed in Normal mode)
- Spectrum reading type
- Area scanning
- Pathlength correction
- Absorbance wavelength calibration (via the Reading Parameters dialog and before performing the read)
- Eject between filter set reads
- Read & Dispense

If dispensing is *required* during a kinetic read, here are two options:

Manual Dispense:

Create a protocol with a long kinetic interval. During an interval, when the reader is idle:

- Push the carrier eject button on the front of the reader to eject the plate
- Perform the dispense using a pipette
- Push the carrier eject button to draw the plate back in
- The read will continue as scheduled

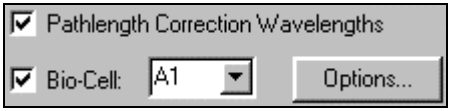
Reader Dispense:

(Injector model only)

Create and run a total of three protocols:

- A Kinetic protocol to start the read. Save results in a .PLA file.
- A "Dispense-only" protocol to dispense the reagent.
- A second Kinetic protocol to complete the kinetics after dispense. Note: Reading parameters in the second protocol must be identical to the first, although kinetic settings can differ.
- Gather the results in a single .PLA file using KC4's Append to Kinetic File feature.

Pathlength Correction



Check Pathlength Correction to normalize absorbances to 1 cm. This option is available for readers that support multiple wavelength reads up to 999 nm.

If Pathlength Correction is enabled, KC4:

1. Calculates the **pathlength** for each well.
2. Calculates the **corrected absorbance** for each well.
3. Reports the **Corrected Data** after Pathlength Correction is applied.

Pathlength Calculation

KC4 calculates each well's pathlength according to the following formula:

$$\frac{(A_{977\ nm} - A_{900\ nm})well}{(A_{977\ nm} - A_{900\ nm})1.0\ cm\ water}$$

- The well is measured at 977 nm and again at 900 nm. KC4 calculates the difference in measurements, then divides that result by the absorbance of water at 1 cm. Note: 977 nm and 900 nm represent default **Test** and **Reference Wavelengths**, respectively. These wavelengths can be changed by clicking the **Options** button.

- KC4 uses a default value of **0.18** for the absorbance of water at 1 cm. This value can be changed by clicking **Options** and defining a different **Absorbance of Solution at 1 cm.**

  Alternatively, click **Bio-Cell** to calculate the absorbance of water at 1 cm value using Bio-Tek's Bio-Cell™ plate. Use the drop-down menu to select the **location** of the cuvette in the plate.

Corrected Absorbance Calculation

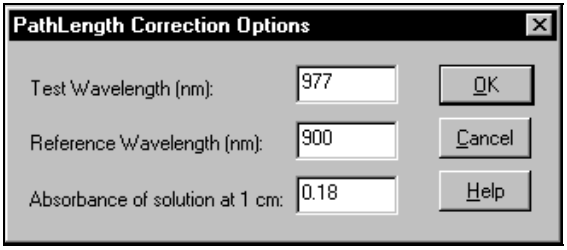
KC4 calculates the **corrected absorbance** in each well according to the following formula:

$$\frac{(A_{wavelength})well}{\text{pathlength of well}}$$

- If a **Blank Plate** is pre-read, and/or if **blank subtraction** is performed, select the advanced protocol option **Raw Data Correction** to control the data used in this formula.

Pathlength Correction Options

Click the **Options** button to change the Test or Reference Wavelength values, or to change the pathlength constant.



- The valid range for the **Test** and **Reference Wavelength** fields is 200 to 999 nm.
- The default **Absorbance of solution at 1 cm** value is 0.18. To use a different constant value when calculating pathlengths, simply enter the desired value.

Reporting the Pathlength Values

KC4 automatically reports the **Corrected Data** after Pathlength Correction is applied. You can easily create a Multi Plate Transformation to report the Pathlength for each well on the plate. Look in the Help system contents under **Results|Data Sets|Pathlengths** for more information.

Plate Type

Important! Be sure to select the appropriate **Plate Type.** Just prior to plate reading, KC4 sends the plate's dimensions to the instrument. This information guides the optical probe(s) to the correct reading positions relative to the microplate. Choosing an inappropriate plate type could adversely affect results.

- Select a **Plate Type** from the drop-down menu. The items presented for selection come from the plate database, which is maintained under **System|Plate Formats.**
- The **First Well** and **Last Well** fields default to include the entire plate. You can change one or both settings to read a partial plate.

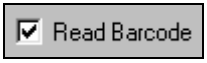
Pre-Read Blank Plate

Check **Pre-Read Blank Plate** to perform two reads at each defined wavelength. The first read is performed on an empty (or blank) plate, and the second on the sample plate. The "Blank Plate" can represent a dry empty plate or a plate filled with solution.

- For **Endpoint** and **Kinetic** reads, the blank plate is read at each wavelength or filter set defined in the protocol.
- For **Spectrum** reads, the blank plate is scanned using the wavelength range defined in the protocol.
- This option is not available if **Scanning** is enabled.

The Blank Plate data is subtracted from the actual microplate data if the **Blank Plate Subtraction** option is selected in the **Raw Data Corrections** dialog (an advanced protocol option).

Read Barcode



If the attached reader is equipped with an internal plate barcode scanner, use the **Read Barcode** feature to read the code when the plate is read. If for any reason the barcode cannot be read, you will be prompted to manually enter the plate barcode.

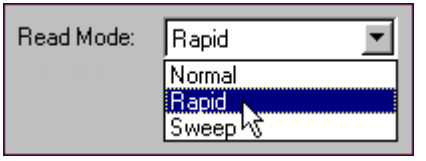
See *Protocol Options* at the end of this chapter to find out how to use the scanned or entered barcode as the data file name.

Read & Dispense

The Synergy™ HT with Injectors provides the ability to dispense fluid into microwells as part of a "Read & Dispense" protocol. To enable this feature, click the **Dispense** checkbox. To define the Dispense, Shake, and/or Read steps, click **Read & Dispense.**

See "Creating a Read & Dispense Protocol" starting on page 119 for detailed instructions.

Read Mode



Some reader models support multiple **Read Modes** for absorbance protocols. The selection of one read mode over another generally depends on the desired outcome of the assay (better precision versus faster plate reading). The available read modes are:

- **Normal** mode is the slowest of the three available modes. After positioning the well over the probe, the instrument waits 100 milliseconds before taking the measurement. This delay is to allow for more complete fluid settling.
- **Rapid** mode is faster than Normal mode because the instrument does not wait before taking the measurement.
- **Sweep** mode is the fastest of the three available modes; the instrument does not stop before taking measurements.

Reader/Read Method

The Reading parameters dialog displays the name of the currently selected **Reader** and the computer port to which it is attached. (This information is configured under System|Readers.)

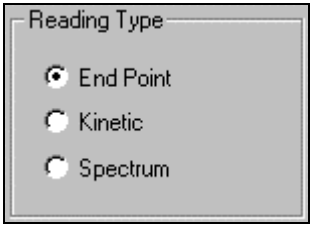
The available Read (or Detection) Methods are reader-dependent. If more than one option is presented, select the desired method by clicking its radio button. This selection instructs KC4 to represent measured values as optical densities (OD), relative fluorescence units (RFU), or relative luminescence units (RLU). It also customizes the Reading parameters dialog to present further options specific to the read method.

Reading Direction

Some readers also support a **Reading Direction** setting. Choose to read in a **horizontal** direction (A1-A12, B1-B12, … H1-H12), or in a **vertical** direction (A1-H1, A2-H2, … A12-H12).

- The faster method for reading a **full plate** is Horizontal.
- The faster method for reading a **single column** is Vertical.

Reading Type



Select a **Reading Type** before defining additional Reading parameters. The Reading Type options **Endpoint** and **Kinetic** are available for all readers supported by KC4. For certain readers, the **Spectrum** reading type is also available.

- During an **Endpoint Read,** every well on the plate is read with one, two, or multiple wavelengths**.**

  If supported by the current reader, the **Scanning** feature is available for endpoint protocols. Click the Scanning… button to define linear or area scanning parameters.
- During a **Kinetic Read,** every well on the plate is read a user-specified number of times with one, two, or multiple wavelengths. Click the **Kinetic…** button to define kinetic reading parameters.
- During a **Spectrum Read,** multiple readings are taken across a wavelength range. The objective is to plot a graph with absorbance or transmittance versus wavelength. Click the **Scales…** button to customize the graphical data displays (optional).
- The Reading Type options for the Synergy™ HT with injectors contains an additional **Dispense** checkbox, to enable the fluid dispense option.

Scales for Kinetic Reads

By default, the **Scales** parameters automatically calculate an appropriate box size to display the kinetic reactions. The default values can be manually overridden by deselecting one or both **Auto** checkboxes.

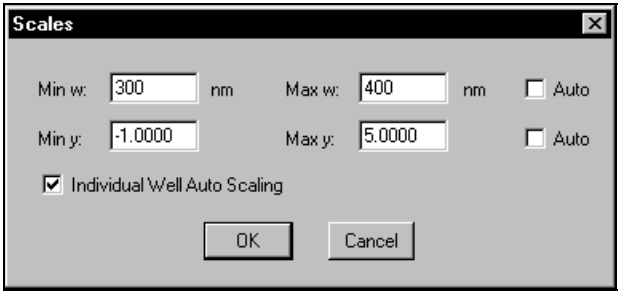
- **Absorbance reads:** min t is automatically set to a zero time value, min y is set to -1.0000 OD, max t is set to the Run Time value, and max y is set to 5.0000 OD.
- **Fluorescence and luminescence reads:** min t and min y are automatically set to zero values, max t is set to the Run Time value, and max y is set to 99999 RFU/RLU (FL600), or to 99998 RFU/RLU (FLx800™ and Synergy™ HT).
- **Individual Well Auto Scaling** individually adjusts the min/max t and min/max y settings for each well to best display its curve. It overrides the settings of the min/max t and min/max y.

  Note: This setting only affects the scale of an individual zoomed well. The kinetic curves display, showing all wells on the plate, is not affected.

Scales for Spectrum Reads

By default, the Scales parameters automatically calculate an appropriate box size to display the spectrum curves. The default values can be overridden manually to change the scaling of the x and y axes.

If the Spectrum reading type is selected in the Reading dialog, click the **Scales…** button to open the Scales dialog:



- The **Scales** parameter defaults are set as follows:

  min w is set to the **Start Wavelength**

  max w is set to the **Stop Wavelength**

  min y is set to -1.0000 OD

  max y is set to 5.0000 OD

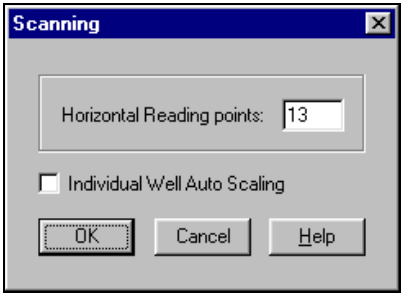
  The default values can be manually overridden by deselecting the **Auto** checkboxes.
- **Individual Well Auto Scaling** individually adjusts the **min/max w** and **min/max y** settings for each well to best display each well's curve. It overrides the settings of the **min/max w** and **min/max y.**

Scanning

If supported by the current reader, the **Scanning** feature is available for Endpoint reads. Some readers support Linear scanning, others support Area scanning:

- When performing a **Linear Scan,** the reader takes multiple measurements in a line across the center of each well. Linear scanning allows you to observe a pattern that may be present in the well bottom, such as an agglutination pattern. Readers that support Linear scanning include the ELx808™ and all PowerWave™ models. **Note for PowerWaveX™ Select:** Linear scanning is supported for the 96-well plate type only.
- When performing an **Area Scan,** the reader takes multiple measurements down and across each well, in a "matrix" format. Area scanning more accurately assesses cellular assays than reading once in the center of the well. Readers that support area scanning include the ELx800™, µQuant™, FLx800™, and Synergy™ HT.
- **Note:** If the Scanning button is dimmed and inaccessible, well scanning cannot be performed with the currently defined plate type. This may be due to a hardware limitation or an unacceptable combination of optic probe size and well diameter.
- Click the **Scanning…** button to define the measurement points:

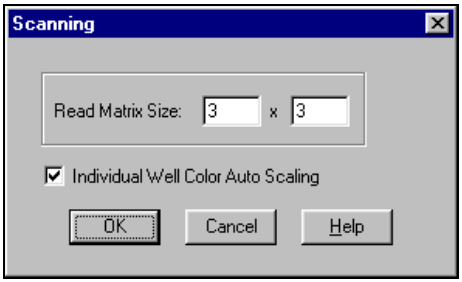
With Linear scanning:



- The **Horizontal Reading Points** setting represents the total number of points to be read across the center of each well. Valid entries are odd integers from 1 to 31.
- **Individual Well Auto Scaling** individually adjusts the **min/max x** and **min/max y** settings for each well to best display its scanning curve. If enabled, this feature overrides the settings of the **min/max x** and **min/max y.**

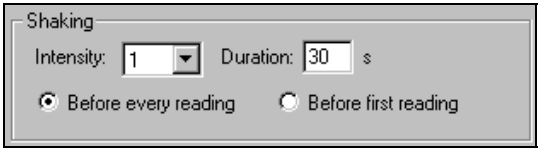
  Note: This feature only affects the scale of an *individual* zoomed well. The scanning curves display, showing all wells on the plate, is not affected.

With Area scanning:



- **Read Matrix Size** represents the number of measurements taken across and down each well. If, for example, the Read Matrix Size is 5 x 5 a total of 25 measurements are taken.
- **Individual Well Color Auto Scaling** determines how the color palette is built when the measurement points are graphically displayed. On *color* monitors and printers, blue shades represent the lowest measurement values, purple shades represents mid-range measurement values, and red shades represent highest measurement values. On *black-and-white* monitors and printers, white represents the lowest measurement values, increasing shades of gray represent increasing measurement values, and black represents the highest measurement values. **Tip:** If the plate is likely to yield values ranging from very low to very high, enable Individual Well (Color) Auto Scaling to help with the visual differentiation between points within each well.

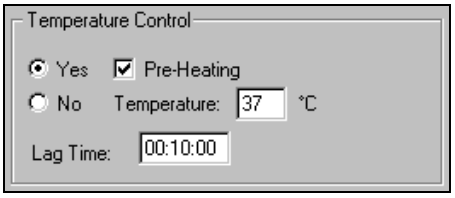
Shaking



If plate shaking is available with the currently selected reader, the **Shaking** parameters are accessible.

- **Endpoint / Spectrum** protocols: Shaking occurs immediately before the plate is read.
- **Kinetic** protocols: Shaking can occur before the first reading only, or before every reading.
- **Intensity:** Acceptable values include:
  - **0** No Shaking
  - **1** Low
  - **2** Medium
  - **3** High
  - **4** Variable (not supported by all readers that support shaking)
- **Duration:** Defines the absolute time period for shaking, in seconds.
- **Before First / Before Every Reading:** Defines when the shaking will occur. For non-kinetic reads, shaking occurs before the first reading. For kinetic reads, shaking can occur before the first read or before every read.

Temperature Control



For readers that support computer-controlled incubation, the **Temperature Control** parameters are accessible:

- Select **Yes** to indicate plate incubation.
- Select **No** to indicate no incubation (default).
- Check **Pre-Heating** to pre-heat the incubator before the plate carrier is drawn in.
- Enter the desired set point **Temperature** in °C.
- Define the **Lag Time** to elapse before the reader initiates the first reading cycle. The reader draws the plate carrier into the reading chamber, waits for the Lag Time to elapse, then reads the plate.
  - Lag Time is available for all reader models, including those that do not support incubation.
  - The valid entry range for most readers is **00:00:00** to **00:59:59** (HH:MM:SS). For the **FL600™,** the range is 00:00:00 to 08:00:00.

Time-Resolved Fluorescence (Synergy™ HT)

If supported by the reader, the **Time-Resolved** option is available when the selected detection method is Fluorescence. If this option is enabled, additional measurement mode parameters become available in the Measurement Options dialog (accessed by clicking the Option button located in the Filters box), to specify the length of time to delay before collecting readings and the length of time during which readings will be taken.

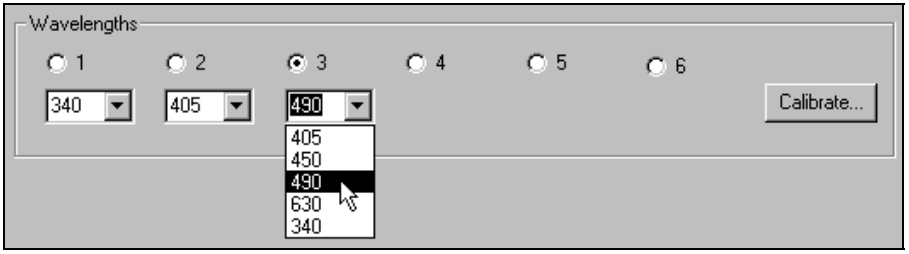
Top Probe Vertical Offset

See “General Options (Synergy HT).”

Wavelengths

For readers equipped with a monochromator (e.g., PowerWave™, PowerWaveX™, µQuant™, Synergy™ in Absorbance mode), the **Wavelengths** set of parameters is available within the Reading parameters dialog.

For **Endpoint** and **Kinetic** reads:



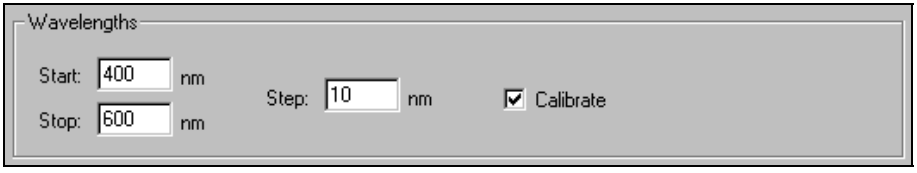
- Click the **radio button** associated with the desired number of wavelengths.
- Click ▼ to open a list of available wavelengths, then click on a wavelength to select it.

  If the desired wavelength is missing from the list, type the value directly into the wavelength field.
- (Optional) If the reader is connected, clicking **Calibrate** will update KC4's wavelength table and the reader's internal wavelength table.

  If KC4's wavelength table already matches the reader's wavelength table, calibration is not necessary.

  If calibration is necessary but the reader is not connected right now, calibration will perform automatically when a plate read begins.

For **Spectrum** reads:



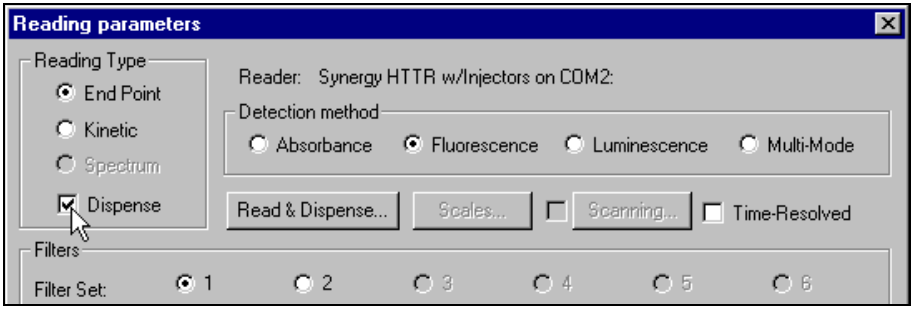
- Define the wavelength range by entering the **Start** and **Stop** values, in nanometers.
- Define the reading interval by entering the **Step** value, in nanometers. For example, if the Start, Stop, and Step values are 300, 600, and 50 nm, respectively, the plate will be read at 300, 350, 400, 450, 500, 550, and 600 nm.
- (Optional) Select **Calibrate:**

  If Calibrate is selected, the reader will always perform calibration at the wavelengths specified in the protocol, just prior to plate reading.

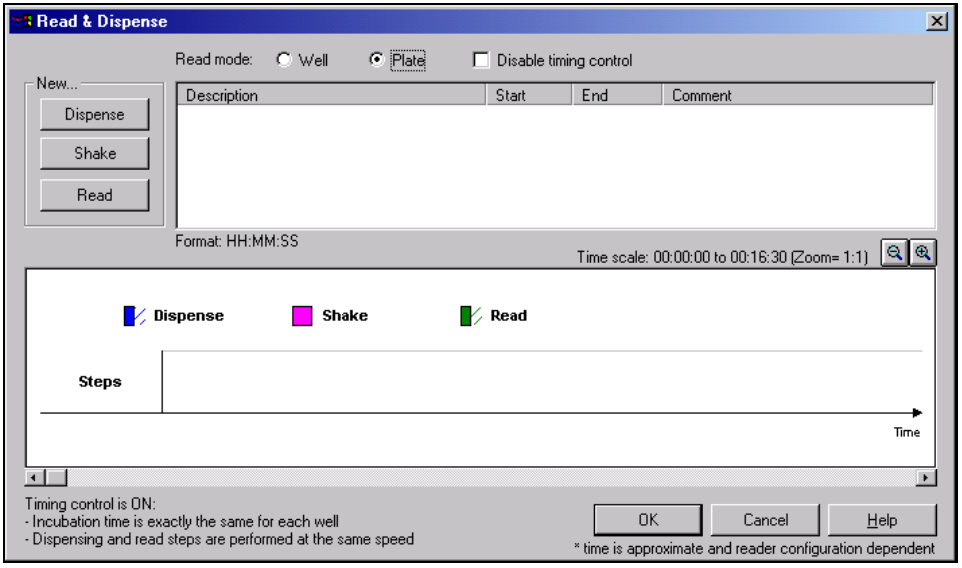
  If Calibrate is *not* selected, the reader will calibrate at only those wavelengths specified in the protocol that have not yet been calibrated since the reader was turned on.

Creating a Read & Dispense Protocol

The Synergy™ HT with injectors provides the ability to dispense fluid into microwells as part of a "Read & Dispense" protocol. To enable this feature, begin by clicking the **Dispense** checkbox within the Reading parameters dialog.



- **Note:** The Dispense option is not available if **Multi-Mode** is selected.
- Define or select additional Reading parameters within this dialog, including Reading Type, Filter Set(s), Plate Type, and Temperature Control.
- Click the **Read & Dispense** button to open the Read & Dispense dialog. Use this dialog to define the Dispense, Shake, and/or Read steps for this protocol.



- Select a **Read Mode:**
  - If **Plate** mode is selected, each step in the sequence is performed on all wells of the plate, step after step. For example, if the step sequence is Dispense / Read, fluid is dispensed into all wells of the plate and then the wells are read. Timings are generally long.

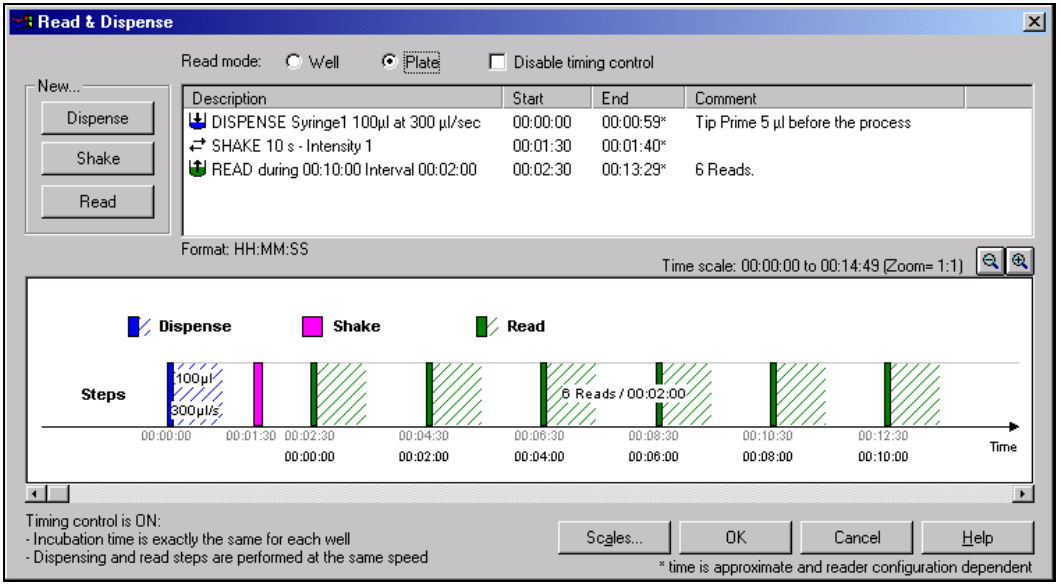
- If **Well** mode is selected, the full sequence of steps is performed on each well, well after well. For example, if the step sequence is Dispense / Read, fluid is dispensed into well A1 and the well is read, fluid is dispensed into well A2 and the well is read, and so on. Timings are generally short (fast).

- The **Time is approximate** indication at the bottom of this dialog means that Start and End times displayed with an asterisk (*) were calculated using appromixation logic. KC4 uses approximation logic when:
  - A step is added with "Shortest, after…" selected.
  - The reader is not communicating with KC4.
  - The reader is unable to validate the Read & Dispense settings.

  In addition, the asterisk will appear if the reader provided a time that can vary based on other reader settings that may change before the protocol is executed (e.g., injector position).

Plate Mode Options

The Dispense, Shake, and Read steps are all available with Plate Mode. In addition, the Timing Control feature is available.


Read & Dispense
Read mode: Well Plate Disable timing control
New...
Dispense
Shake
Read
Description Start End Comment
DISPENSE Syringe1 100µl at 300 µl/sec 00:00:00 00:00:59* Tip Prime 5 µl before the process
SHAKE 10 s - Intensity 1 00:01:30 00:01:40*
READ during 00:10:00 Interval 00:02:00 00:02:30 00:13:29* 6 Reads.
Format: HH:MM:SS
Time scale: 00:00:00 to 00:14:49 (Zoom= 1:1)
Dispense
Shake
Read
Steps
100µl
300µl/s
6 Reads / 00:02:00
00:00:00 00:01:30 00:02:30 00:04:30 00:06:30 00:08:30 00:10:30 00:12:30
00:00:00 00:02:00 00:04:00 00:06:00 00:08:00 00:10:00
Time
Timing control is ON:
- Incubation time is exactly the same for each well
- Dispensing and read steps are performed at the same speed
Scales... OK Cancel Help
* time is approximate and reader configuration dependent

- **Timing Control** is enabled by default. To disable Timing Control, check the box.

  When Timing Control is *enabled*, all steps are processed in the same way with regard to timing:
  - Incubation time is exactly the same for each well.
  - Dispense and Read steps are performed at the same pace.

  When Timing Control is *disabled*, all steps are performed as quickly as possible:
  - Incubation time may vary across the microplate.
  - Dispense and Read steps may be performed at different speeds.

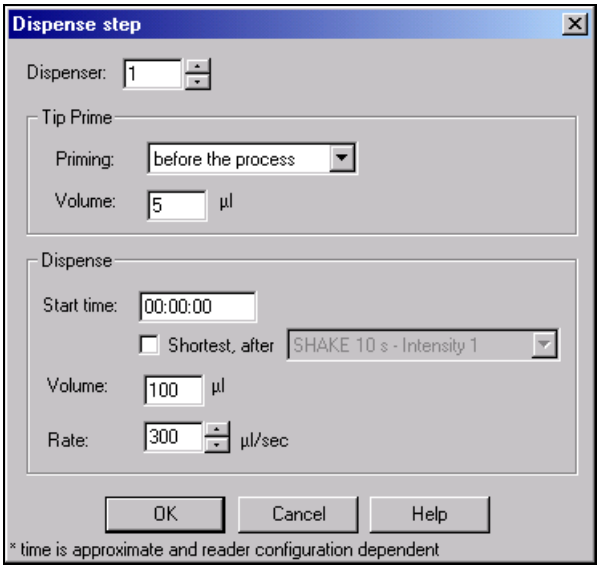
- Click the Dispense, Shake, and Read buttons to create new **steps.**
  - Up to nine (9) steps may be defined.
  - Steps cannot overlap. They must be performed one after another.
  - With Endpoint protocols, only one Read step may be defined.
  - Endpoint protocols do not *require* a Read step (the protocol can be "Dispense-only").
  - Kinetic protocols require at least one Read step. **Note:** If the protocol contains two or more Read steps, the Kinetic Interval is represented as 'Uneven' on reports.
  - A Kinetic Read step with Shake During Read enabled counts as *two* steps.
- The steps are **listed** in the order in which they will be executed.
  - The **Description** column provides key information about the step, such as volume, rate, or intensity.
  - The **Start** column indicates the time the step will begin.
  - The **End** column indicates the time the step will complete.
  - The **Comment** column contains additional information about the step, such as tip prime settings.
  - To **edit** a step, highlight it and double-click or click the right mouse button and select Edit. To **delete** a step, highlight it and press the Delete key or click the right mouse button and select Delete.
- The steps are also displayed **graphically** to show how they will be executed on the plate over time.
  - **Dispense** step: The solid area represents the action on the first well. Cross hatches represent the time necessary to process other wells on the plate.
  - **Shake** step: The solid area represents the action on the entire plate.
  - **Read** step: The solid area represents the action on the first well (and for each kinetic interval, if this is a kinetic protocol). Cross hatches represent the time necessary to process other wells on the plate.

  Time indicators:

  - A **Process** time indicates the time at which a particular **step** will begin, relative to the start time of the very first step in the operation. Process times are displayed in grey (light) text.
  - (Kinetic protocols only.) A **Kinetic** time indicates the time at which a **reading** begins, relative to the start time of the very first read in the operation (which is always 00:00:00). Kinetic times are displayed underneath the Process times in black (dark) text. Note that in a kinetic protocol, each read step will have a Process time and a Kinetic time associated with it.

Dispense Step Parameters

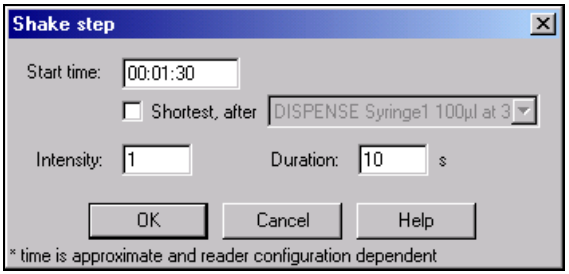
Use this dialog to create or modify a **Dispense** step:



- Select the number of the **Dispenser** to be used for this step. The numbers available for selection correspond to the numbers on the dispense box.
- Select a **Tip Prime** option. Tip priming is performed in a small, removable priming trough located in the left rear. The purpose of tip priming is to compensate for any fluid loss at the dispense tip due to evaporation since the last dispense. The trough holds up to 1 ml of liquid and must be periodically emptied and cleaned by the user. KC4 must then be "told" that the trough has been emptied (via the System > Reader Control dialog). If the trough overflows, refer to the Synergy™ HT Operator's Manual for decontamination instructions.
  - **None:** No prime.
  - **Before the process:** Prime the tip with fluid once before the entire process begins.
  - **Before the dispense:** Prime the tip with fluid before each dispense to a well.
- If tip priming will be performed, enter the **Volume** in microliters. The valid range is 5 to 20. The dispense rate will be the same as that selected for the well dispense.
- Set the **Start Time** for the step. Enter the time manually (HH:MM:SS), or check **Shortest** and select the step immediately after which this Dispense step shall be performed.
- Enter the dispense **Volume,** in microliters. The valid range is 5 to 1000.
- Select the dispense **Rate** in microliters per second. The default rate is the maximum available for the attached instrument.
- Click **OK** to save the settings and add the Dispense step to the list.

Shake Step Parameters

Use this dialog to create or modify a **Shake** step:

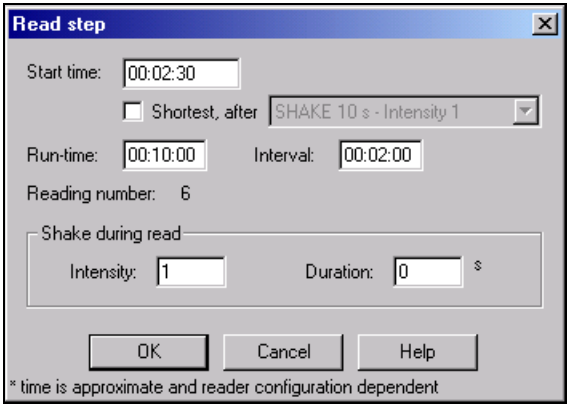


- Set the **Start Time** for the step. Enter the time manually (HH:MM:SS), or check **Shortest** and select the step immediately after which this Shake step shall be performed.
- Specify the shake **Intensity.** Acceptable values include:

  1 Low
  2 Medium
  3 High
  4 Variable
- Specify the shake **Duration** in seconds.
- Click **OK** to save the settings and add the Shake step to the list.
- **Note:** Shaking can also be defined as a part of the Read step if repeated shaking is required between each kinetic reading interval.

Read Step Parameters

Use this dialog to create or modify a **Read** step:



- Set the **Start Time** for the step. Enter the time manually (HH:MM:SS), or check **Shortest** and select the step immediately after which this Read step shall be performed.
- Kinetic protocols only:
  - Specify the **Run Time.** This is the length of time from the start of the first kinetic read to the start of the last kinetic read.

- Specify the time **Interval** for repeat reading of the plate, section of wells, or individual well. The maximum valid Interval is equal to the current Run Time.
- KC4 automatically calculates the **Reading Number** by dividing the Run Time by the Interval and adding 1.
- If Shaking before each kinetic read is required, specify the shake **Intensity** and then specify the shake **Duration** in seconds (a Duration of 0 means no shaking will occur).

  Note: Shaking can also be defined as an individual step outside of the Read step, using the Shake button in the Read & Dispense dialog.

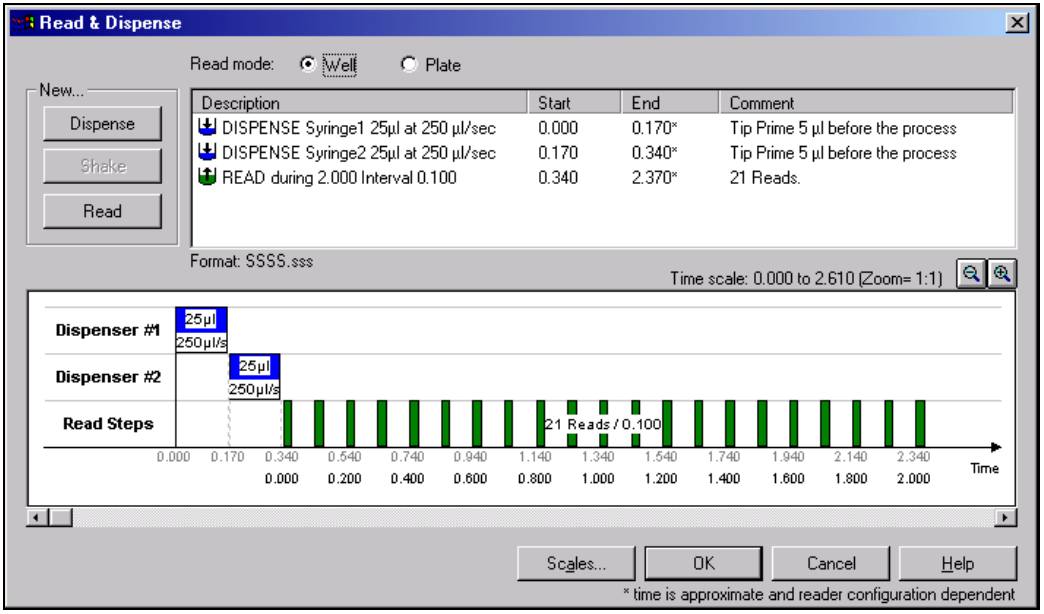
  Acceptable Intensity values include:

  1 Low
  2 Medium
  3 High
  4 Variable

- Click **OK** to save the settings and add the Read step to the list.

Well Mode Options

The Dispense and Read steps are available with Well Mode. The Timing Control feature is not available.



- Click the Dispense and Read buttons to create new **steps.**
  - Up to nine (9) steps may be defined.
  - Shaking is not permitted in Well Mode.
  - Endpoint protocols require one Read step.

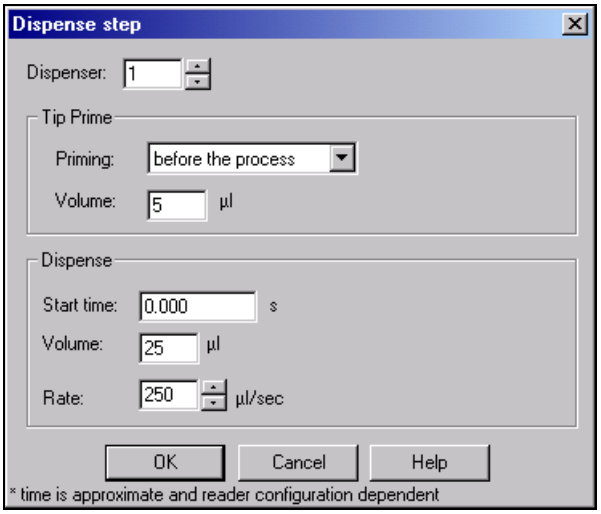
- Kinetic protocols require at least one Read step. **Note:** If the protocol contains two or more Read steps, the Kinetic Interval is represented as 'Uneven' on reports.
- Dispense steps cannot overlap. (Only one dispenser can be in use at a time.)
- A Read step can overlap a Dispense step. For example, the dispense may occur during a kinetic read, to show the rate of change immediately following the injection of fluid. (This can also be accomplished by creating two Read steps, one immediately before and one immediately after the Dispense step).

- The steps are **listed** in the order in which they will be executed.
  - The **Description** column provides key information about the step, such as volume, rate, or intensity.
  - The **Start** column indicates the time the step will begin.
  - The **End** column indicates the time the step will complete.
  - The **Comment** column contains additional information about the step if needed.
  - To **edit** a step, highlight it and double-click or click the right mouse button and select Edit. To **delete** a step, highlight it and press the Delete key or click the right mouse button and select Delete.
- The steps and are also displayed **graphically** to show how they will be executed on each well over time. Dispenser #1, Dispenser #2, and Read steps are shown in parallel.
  - **Dispense** step: The solid area represents the execution of the Dispense for one well.
  - **Read** step: The solid area represents the execution of the Read step for one well.

  Time indicators:
  - A **Process** time indicates the time at which a particular **step** will begin, relative to the start time of the very first step in the operation. Process times are displayed in grey (light) text.
  - (Kinetic protocols only.) A **Kinetic** time indicates the time at which a **reading** begins, relative to the start time of the very first read in the operation (which is always 00:00:00). Kinetic times are displayed underneath the Process times in black (dark) text. Note that in a kinetic protocol, each read step will have a Process time and a Kinetic time associated with it.

Dispense Step Parameters

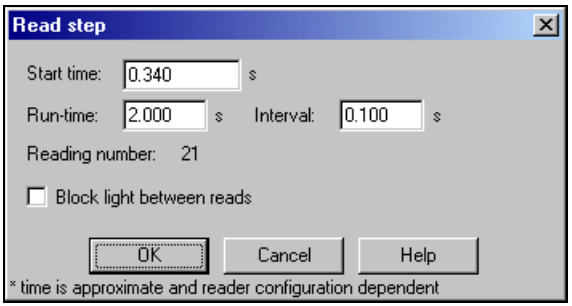
Use this dialog to create or modify a Dispense step:



- Select the number of the **Dispenser** to be used for this step. The numbers available for selection correspond to the numbers on the dispense box.
- Select a **Tip Prime** option. Tip priming is performed in a small, removable priming trough located in the left rear of the carrier. The purpose of tip priming is to compensate for any fluid loss at the dispense tip due to evaporation since the last dispense. The trough holds up to 1 ml of liquid and must be periodically emptied and cleaned by the user. KC4 must then be "told" that the trough has been emptied (via the System > Reader Control dialog). If the trough overflows, refer to the Synergy™ HT Operator's Manual for decontamination instructions.
  - **None:** No prime.
  - **Before the process:** Prime the tip with fluid once before the entire process begins.
  - **Before each well:** Prime the tip with fluid before dispensing to each well.
- If tip priming will be performed, enter the **Volume** in microliters. The valid range is 5 to 20.
- Set the **Start Time** for the step. Enter the time manually (SSSSS.sss), or check **Shortest** and select the step immediately after which this Dispense step shall be performed.
- Enter the dispense **Volume,** in microliters. The valid range is 5 to 1000.
- Select the dispense **Rate** in microliters per second. The default rate is the maximum available for the attached instrument.
- Click **OK** to save the settings and add the Dispense step to the list.

Read Step Parameters

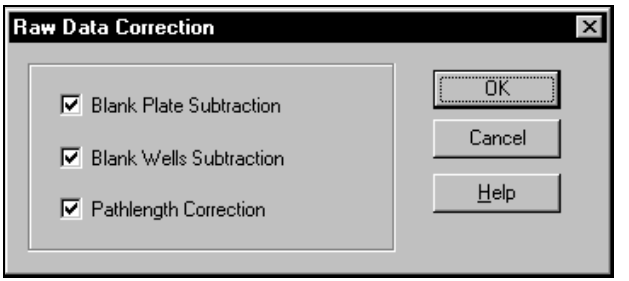
Use this dialog to create or edit a Read step:



- Enter the **Start Time** in seconds (sssss.sss).
- Kinetic protocols only:
  - Specify the **Run Time.** This is the length of time from the start of the first kinetic read to the start of the last kinetic read.
  - Specify the time **Interval** for repeat reading of the plate, section of wells, or individual well. The maximum valid Interval is equal to the current Run Time.
  - KC4 automatically calculates the **Reading Number** by dividing the Run Time by the Interval and adding 1.
- (Optional) For non-TR Fluorescence reads, check **Block light between reads** to block the light between measurements to inhibit photo bleaching.
  - **Single filter set protocols:** If light blocking is enabled, a blocking filter (Plug) must be placed in the excitation filter wheel in one of the two positions next to the excitation filter that is specified in the protocol.
  - **Dual filter set protocols:** If light blocking is enabled, two blocking filters (Plugs) must be placed next to each other in the excitation filter wheel.
- Click **OK** to save the settings and add the Read step to the list.

Raw Data Correction

Raw data, or the optical density or fluorescence unit values retrieved from a microplate reader, can be **corrected** using Blank Plate Subtraction, Blank Wells Subtraction, and/or Pathlength Correction. The advanced protocol option **Raw Data Correction** controls the use of these correction options.



- **Blank Plate Subtraction** is accessible if "Pre-Read Blank Plate" is selected in the Reading parameters dialog.
- **Blank Wells Subtraction** is accessible if one or more blank wells are defined under Plate Layout.
- **Pathlength Correction** is accessible if it is selected in the Reading parameters dialog.
- Raw Data Correction calculations are performed in the order Blank Plate Subtraction, Blank Wells Subtraction, then Pathlength Correction.

The following **example** describes the plate processing and calculation steps performed when a protocol specifies Pre-Read Blank Plate, Pathlength Correction with Bio-Cell, and the plate is configured with blank wells. This example is for explanation only, the typical application does not use all three raw data correction options.

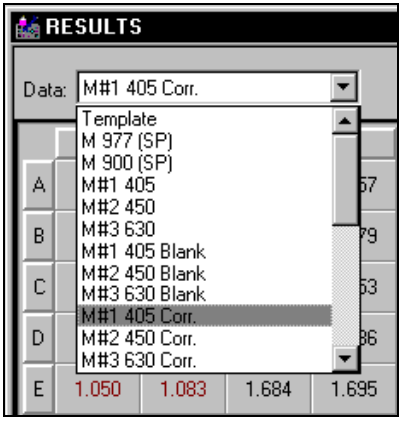
Processing steps:

1. The Blank Plate is read at every wavelength defined in the Reading parameters dialog.
2. The Bio-Cell is read at 977 nm and 900 nm.
3. The Sample Plate is read at 977 nm and 900 nm.
4. The Sample Plate is read at every wavelength defined in the Reading parameters dialog.

Calculation steps performed for each wavelength defined in the Reading parameters dialog:

1. The basis of calculations begins with the raw Sample Plate readings.
2. The Blank Plate readings are subtracted from the Sample Plate readings. The result of this calculation becomes the basis for step 3.
3. The mean of the blank wells is calculated and subtracted from every well on the plate. The result of this calculation becomes the basis for step 4.
4. The value in each well is multiplied by the difference in readings of the Bio-Cell at 977 nm and 900 nm. The result of this calculation becomes the basis for step 5.
5. The value in each well is divided by the difference in readings of the Sample Plate at 977 nm and 900 nm. The result of this calculation is the final corrected value.

The image below shows the **data sets** available following a three-wavelength read with Blank Plate Subtraction, Blank Wells Subtraction, and Pathlength Correction enabled:



- **M 977 (SP)** and **M 900 (SP)** contain the values of the Sample Plate read at 977 nm and 900 nm, respectively (necessary for pathlength correction calculation).
- Measurement numbers **(M#1, M#2, M#3)** correspond with the wavelength selection order in the Reading parameters dialog.
- **M#1 405, M#2 450,** and **M#3 630** contain the raw Sample Plate values at 405 nm, 450 nm, and 630 nm.
- **M#1 405 Blank, M#2 450 Blank,** and **M#3 630 Blank** contain the values calculated by subtracting the Blank Plate readings at the specified wavelength from the raw Sample Plate readings at the specified wavelength. These values do *not* represent **Blank Well Subtraction.**
- **M#1 405 Corr., M#2 450 Corr.,** and **M#3 630 Corr.** contain the final values after all Raw Data Correction options have been applied.

Blank Plate Subtraction

With most readers, the Pre-Read Blank Plate option is available within the Reading parameters dialog. If this option is selected, a **blank plate** is read before the sample plate, at the wavelengths defined in the protocol. KC4 then calculates the difference in measurements between the two sets of data (sample - blank). **Note:** The blank plate can represent an empty dry plate or a plate filled with solution for blanking.

If **Blank Plate Subtraction** is enabled:

- **Endpoint** read: For each wavelength defined in the protocol, KC4 subtracts the value of each well of the blank plate from the corresponding well of the sample plate.
- **Kinetic** read: For each wavelength defined in the protocol, within each kinetic read, KC4 subtracts the value of each well of the blank plate from the corresponding wells of the sample plate.
- **Spectrum** read: At each wavelength read within the defined spectrum range, KC4 subtracts the value of each well of the blank plate from the corresponding well and wavelength of the sample plate.

Blank Wells Subtraction

Within any protocol, if one or more wells are defined as 'Blank' under Plate Layout, the **Blank Wells Subtraction** Raw Data Correction option automatically becomes enabled. When this option is enabled:

- **Endpoint** read: For each wavelength defined in the protocol, the average of the blank well(s) is subtracted from every well on the plate.

  Note: For readers that do not accept multiple (> 2) wavelengths, if a dual-wavelength read is defined, KC4 automatically calculates the Delta OD first. The average of the Delta OD values in the blank wells is then subtracted from every well on the plate.

- **Kinetic** read: For each wavelength defined in the protocol, within each kinetic read, the average of the blank wells is subtracted from every well on the plate.

  Note: For readers that do not accept multiple (> 2) wavelengths, if a dual-wavelength read is defined, KC4 automatically calculates the Delta OD first. Within each kinetic read, the average of the Delta OD values in the blank wells is then subtracted from every well on the plate.

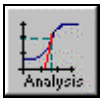
- **Spectrum** read: A blank average is calculated for each wavelength in the spectrum reading range. The blank average for each wavelength is then subtracted from the absorbance read at the corresponding wavelength in each well.

Pathlength Correction

With readers that support multiple-wavelength reads up to 999 nm, the Pathlength Correction option is available within the Reading parameters dialog. If this option is selected, absorbances are normalized to 1 cm.

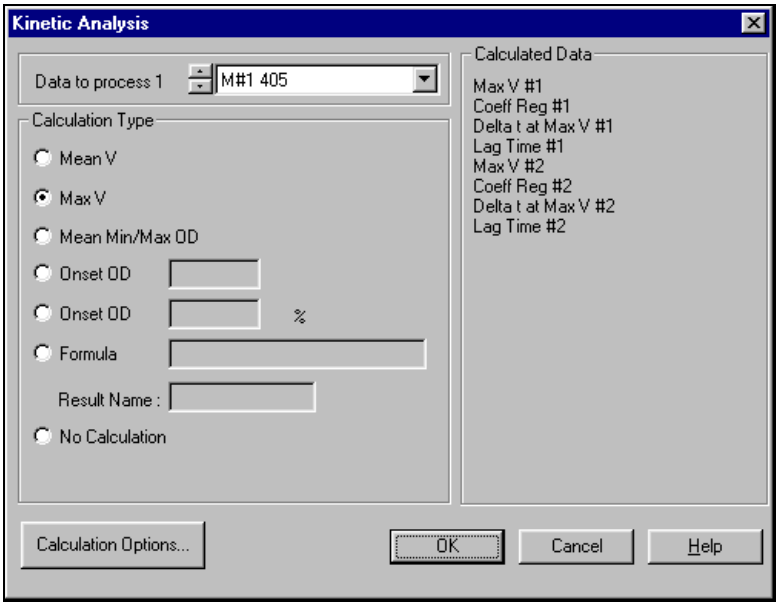
If Pathlength Correction is enabled, KC4 first calculates the **pathlength** for each well. The absorbance in each well is then divided by the pathlength, resulting in the **corrected absorbance.** See "Pathlength Correction" in the *Reading Parameters Reference* section to learn more.

Kinetic Analysis



Use **Kinetic Analysis** to select pre-data reduction parameters for kinetic reads.

This option becomes available within the Protocol menu when the **Kinetic** read method is selected.



Data to Process

This option allows the selection of the type of data to be analyzed, i.e., the measured response value for kinetic analysis. The drop-down list displays the available data types.

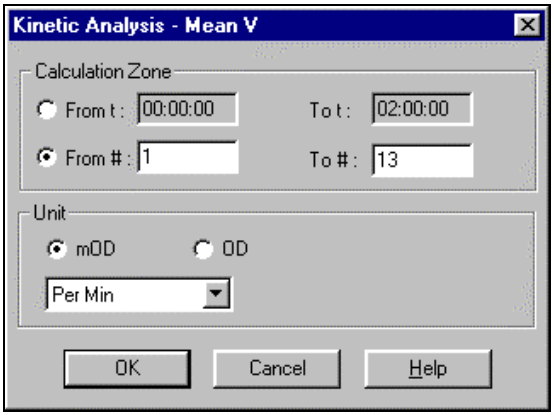
Typical uses of this feature:

- To **blank** data prior to the kinetic slope calculation. First, define one or more blank wells in the Plate Layout, and make sure Blank Wells Subtraction is enabled in the Raw Data Correction dialog. Then, select '<wavelength> Corr.' from the Kinetic Analysis **Data to Process** pull-down list.
- With some readers it is possible to perform kinetic reading with multiple filters or wavelengths. In this case, two levels of **Data to Process** options can be selected (e.g., 'M#1 405' and 'M#2 550') to perform and compare kinetic calculations.

  Note: When two data sets are selected, the kinetic curves are automatically overlaid in the kinetic graphs and in the kinetic zooms.

Mean V

Mean V is the calculated value of the mean slope. KC4 determines the **mean slope** by calculating a linear regression on points that are in the calculation zone. To set the calculation zone, select Mean V, then click on **Calculation Options.**



Calculation Zone

Typically, the Calculation Zone is adjusted to ignore erroneous data point(s) generated at the beginning of a kinetic assay due to assay "noise".

- **From t / To t :** Defines a calculation zone based on *time*. The default values span the defined kinetic Run Time.
- **From # / To # :** Defines a calculation zone based on *number of reads*. The default values include all reading points.

Units

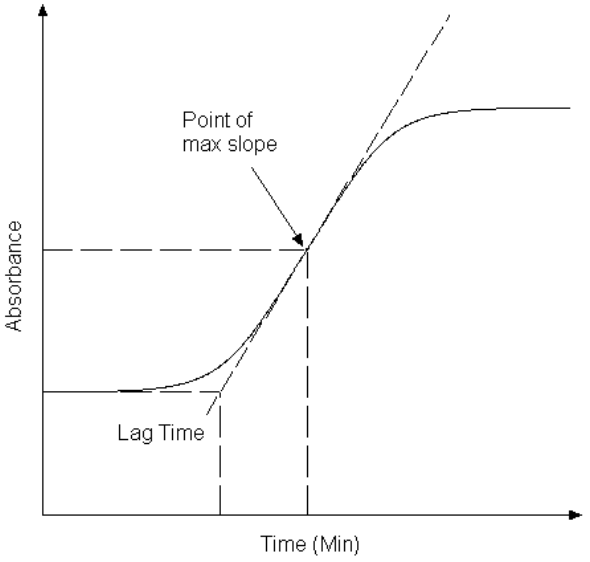
- **mOD** or **OD** dependent on kinetic dynamic range.
- **Per sec** or **Per sec2** dependent on rate order.
- **Per min** or **Per min2** dependent on rate order.
- **Per hour** or **Per hour2** dependent on rate order.

Max V

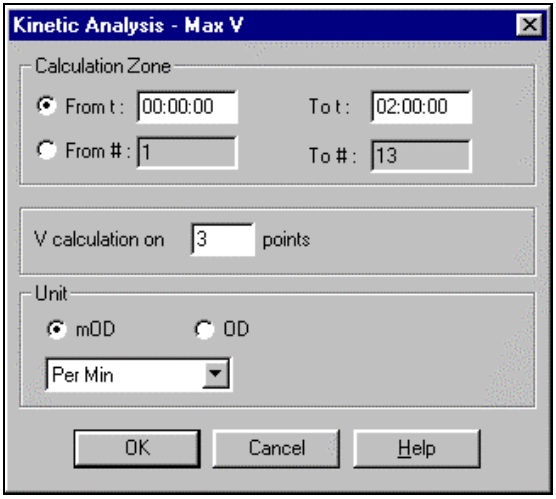
With **Max V,** the calculated value is the maximum slope. KC4 calculates the **maximum slope** as follows:

- Starting at the first point in the calculation zone, KC4 evaluates *n* points and calculates the slope among these *n* points.
- Next, KC4 repeats the operation, starting at the second point in the calculation zone, and repeats again, starting at the third point, and so on.
- KC4 then compares all calculated slopes to determine the maximum slope.
- KC4 will register the **Delta t** time in the middle of the point where the **V max** is calculated.

KC4 also calculates the kinetic **Lag Time,** which is the time intercept between the line of maximum slope of the propagation phase and the absorbance baseline at time = 0, as illustrated below:



By default, the calculation zone starts at time zero. To change the calculation zone, select Max V, then click **Calculation Options.**



Calculation Zone

Typically, the Calculation Zone is adjusted to ignore erroneous data point(s) generated at the beginning of a kinetic assay due to assay "noise".

- **From t / To t :** Defines a calculation zone based on *time*. The default values span the defined kinetic Run Time.
- **From # / To # :** Defines a calculation zone based on *number of reads*. The default values include all reading points.

V calculation on *n* points

- This option defines the number of successive points to be included in the calculation of the maximum slope.
- The minimum number of points is 2. The maximum number of points is the total number of points within the calculation zone.
- Only the points defined within the calculation zone are used.

Units

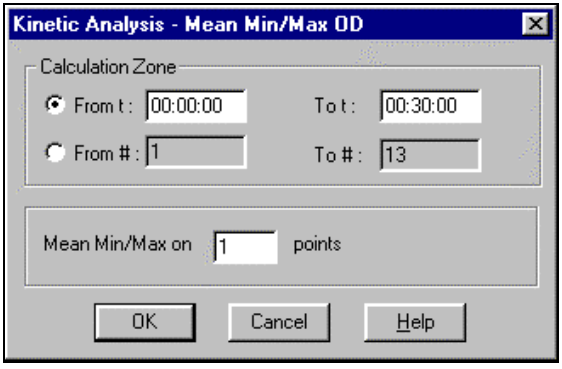
- **mOD** or **OD** dependent on kinetic dynamic range.
- **Per sec** or **Per sec2** dependent on rate order.
- **Per min** or **Per min2** dependent on rate order.
- **Per hour** or **Per hour2** dependent on rate order.

Mean Min/Max

The **Mean Min OD** is the mean minimum OD based on *n* points. The **Mean Max OD** is the mean maximum OD based on *n* points. KC4 calculates the **Mean Min** and **Mean Max ODs** as follows:

- Starting at the first point in the calculation zone, KC4 evaluates *n* points and calculates the mean among these *n* points.
- Next, KC4 repeats the operation, starting at the second point in the calculation zone, and repeats again, starting at the third point, and so on.
- KC4 then compares all of the calculated means to determine the minimum and maximum of these values.
- KC4 also calculates the Delta t at Min/Max OD.
- **Note:** For fluorescence and luminescence reads, KC4 performs calculations based on fluorescence units (Mean Min/Max RFU) and luminescence units (Mean Min/Max RLU), respectively.

To set the calculation zone, select Mean Min/Max OD, then click **Calculation Options.**



Calculation Zone

Typically, the Calculation Zone is adjusted to ignore erroneous data point(s) generated at the beginning of a kinetic assay due to assay "noise".

- **From t / To t :** Defines a calculation zone based on *time*. The default values span the defined kinetic Run Time.
- **From # / To # :** Defines a calculation zone based on *number of reads*. The default values include all reading points.

Mean Min/Max on *n* points

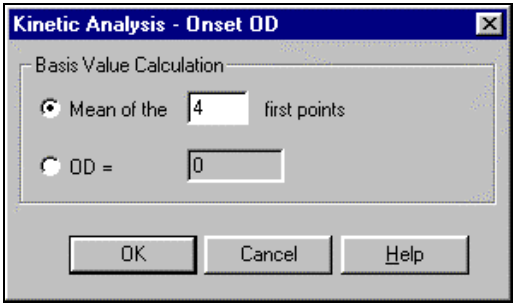
- Selects the number of points to be included in the average calculation.

Onset OD / Onset OD %

The calculated value is the time when the OD reaches a set decrease/increase (Onset OD), that can be defined by an absolute value or a relative value (%), compared with a reference OD (the Basis Value). You can either manually enter this value, or have KC4 calculate it (mean of the *n* first points). By default *n*=1, thus the Basis Value is the OD of the first point.

- KC4 returns the time for each well to pass the **Onset OD** value.
- **Note:** For fluorescence and luminescence reads, KC4 performs calculations based on fluorescence units (Onset RFU) and luminescence units (Onset RLU), respectively.

To set the calculation zone, select Onset OD or Onset OD %, then click **Calculation Options.**



Basis Value Calculation

- **Mean of the *n* first points:** For relative determination of the Basis Value.
- **OD = :** For absolute determination of the Basis Value.

  Examples:

  Onset OD=0.5, Basis Value=Mean of first 2 points:
  KC4 calculates the time that is required for the OD to increase by 0.5 OD, compared with the mean of the 2 first points of the kinetics.

  Onset OD=15%, Basis Value=Mean of the first point:
  KC4 calculates the time that is required for the OD to increase by 15%, compared with the first point of the kinetics.

  Onset OD=1.6, Basis Value=0:
  KC4 calculates the time that is required for the OD to reach the value of 1.6 (Basis Value + Onset OD).

Formula

Select **Formula** to define a formula to calculate a value using data from individual reading points from the same well. Designate an individual reading point using the identifier **R*n*,** where *n* represents the reading number, then enter a **Result Name** in which to store the formula results. This name can be used as a Data-In setting in **Transformations.** Formula examples:

- The formula **R10-R5** calculates the difference in readings between data points 10 and 5.
- The formula **R#** (e.g., R1) extracts a single reading from each well for endpoint data reduction.

No Calculation

This option, if selected, overrides any calculations on the kinetic data, and allows the display of kinetic graphics without data reduction.

- **No Calculation** is typically used to display overlaid curves.

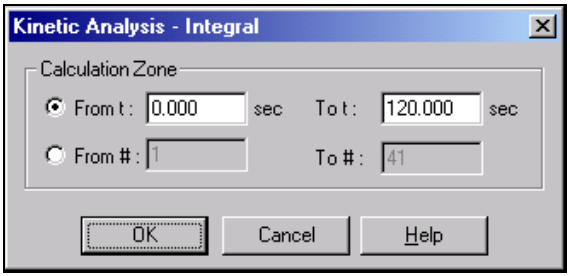
Integral

Select **Integral** to calculate the area under the curve according to the trapezoidal method, shown below:

$$\text{Integral} = \sum_{i=1}^{i=n-1} ((y_i + y_{i+1}) / 2) \times (x_{i+1} - x_i)$$

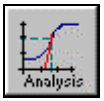
Where y = measurement value and x = read point value.

By default, the calculation zone spans the full Run Time and includes all of the reading points defined in the protocol. Click **Calculation Options** to change the calculation zone:



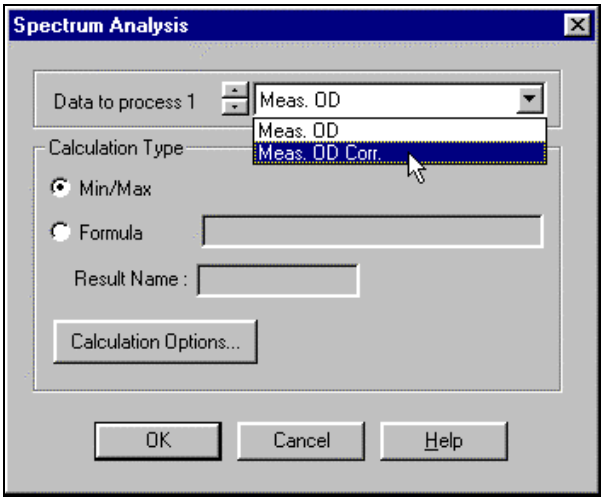
- **From t / To t :** Defines a calculation zone based on *time*. The default values span the defined kinetic Run Time.
- **From # / To # :** Defines a calculation zone based on *number of reads*. The default values include all reading points.

Spectrum Analysis



KC4 provides the option for performing additional calculations on spectral data. This is available for **Spectrum** reading types only.

Open the **Spectrum Analysis** dialog by selecting **Protocol|Spectrum Analysis:**



Data to Process

Select up to two levels of data to be used in calculations.

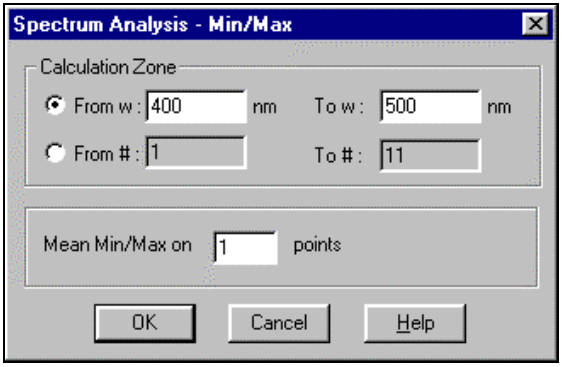
- When two data sets are selected, the spectrum curves are automatically overlaid in the spectrum graphs and in the spectrum zooms.

Min/Max

Select **Min/Max** to determine the **minimum** and **maximum** values for the points defined within the calculation zone. This is the default setting. Upon completion of a read, the following is reported for each well:

- Minimum OD and Wavelength at Minimum OD
- Maximum OD and Wavelength at Maximum OD

If the Calculation Type is set for **Min/Max,** certain calculation parameters may be customized. Access the **Spectrum Analysis - Min/Max** dialog by clicking the Calculation Options button.



- **From w** and **To w** allow the specification of a range of wavelengths upon which to perform calculations. The default values correspond with the **Start** and **Stop** values in the Protocol|Reading Parameters dialog.
- **From #** and **To #** allow the specification of a range of read numbers upon which to perform calculations. The default values correspond with the total number of **wavelengths** to be read, based on the Start, Stop, and Step values in the Protocol|Reading Parameters dialog.

  Example: Start/Stop/Step values set to 400/600/10, respectively. The default From # and To # values are 1 and 21, indicating a calculation zone from 400-600 nm. Setting From # to 2, and To # to 18 would change the calculation zone to 410-570 nm.
- **Mean Min/Max** specifies the number of successive wavelength readings to be averaged prior to the determination of the Minimum and Maximum OD values.

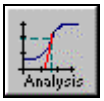
Formula

Check the **Formula** radio button to define a formula to structure calculations by a formula. Enter the formula into the long field.

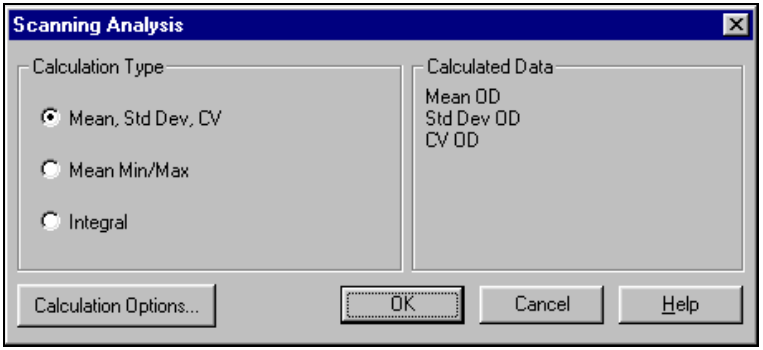
Example: The formula **W1+W2+W3** calculates the sum of the ODs for the readings at wavelengths 1, 2, and 3. If the plate is read from 400 to 410 nm in 2 nm increments, W1 will correspond with the OD at 400 nm, W2 with the OD at 402 nm, and W3 with the OD at 404 nm.

- Enter a **Result Name** in which to store the formula results. This name can be used as a **Data-In** variable in Transformations.
- **Mean Min/Max** specifies the number of successive wavelength readings to be averaged prior to the determination of the Minimum and Maximum OD values.

Scanning Analysis



If linear scanning is selected in an endpoint protocol, select **Protocol|Scanning Analysis** to specify data reduction options:

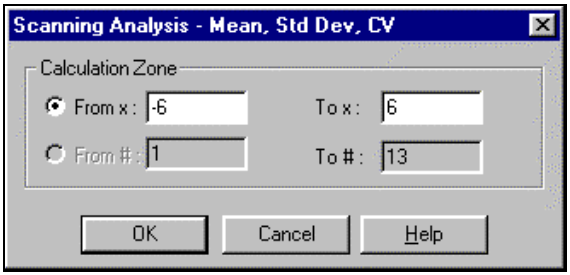


- Select the **Calculation Type** to be performed on each scanned well.
- Click the **Calculation Options** button to define the calculation zone. By default, the calculation zone includes all of the Horizontal Reading Points defined in the protocol.

Mean, Std Dev, CV

Select **Mean, Std Dev, CV** to calculate and report the Mean, Standard Deviation, and Coefficient of Variation for all points in the calculation zone.

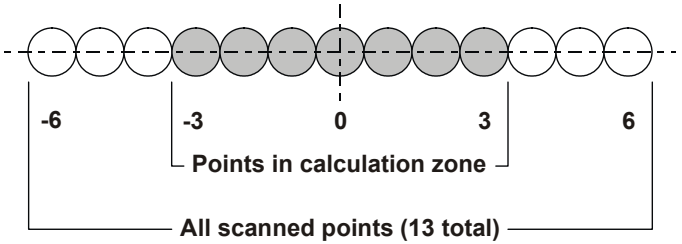
By default, the calculation zone includes all of the Horizontal Reading Points defined in the protocol. To change the calculation zone, click **Calculation Options.**



- Currently, only the "From x: / To x: " method for determining the calculation zone is supported.

  By default, **From x:** represents the outermost scanned point to the *left* of center (0), and **To x:** represents the outermost scanned point to the *right* of center. These values can be changed to base calculations on a subset of all scanned points.

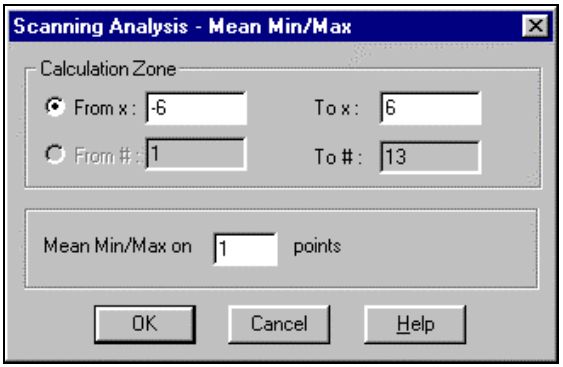
In the example below, the number of Horizontal Reading Points in the protocol is 13. The default From x: and To x: settings are - 6 and 6, respectively. To change the calculation zone to include only the three points to the left and right of center (0), set From x: to -3 and To x: to 3.



Mean Min/Max

Select **Mean Min/Max** to determine and report the Mean Minimum and Mean Maximum measurement values based on *n* points.

By default, the calculation zone includes all of the Horizontal Reading Points defined in the protocol, and the *n* value is 1. To change the calculation zone and/or the *n* value, click **Calculation Options.**



- Currently, only the "From x: / To x: " method for determining the calculation zone is supported. See "Mean, Std Dev, CV" for more information.
- The default number of points (*n*) used to calculate the Mean Minimum and Mean Maximum values is 1. KC4 calculates the Mean Minimum and Mean Maximum values as follows:
  - Starting at the first point in the calculation zone, KC4 evaluates *n* points and calculates the mean.
  - KC4 repeats the operation, starting at the second point in the calculation zone, then repeats again starting at the third point, and so on.
  - KC4 then compares all mean values to determine the minimum and maximum values.

Integral

Use the **Integral** calculation option to provide the area under the curve according to the trapezoidal method, shown below:

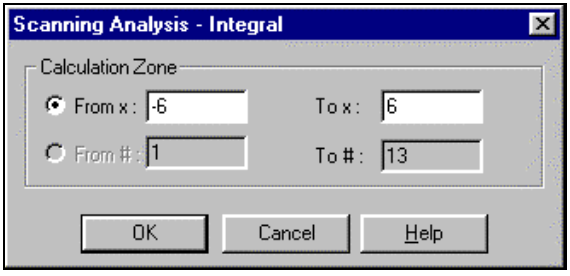
$$\text{Integral} = \sum_{i=1}^{i=n-1} ((y_i + y_{i+1}) / 2) \times (x_{i+1} - x_i)$$

Where y = absorbance and x = scan point value.

Note that in linear scanning, (x_{i+1} - x_i) is equal to 1, and thus the calculation formula is reduced to:

$$\text{Integral} = \sum_{i=1}^{i=n-1} y_i - ((y_1 + y_n) / 2)$$

By default, the calculation zone includes all of the Horizontal Reading Points defined in the protocol. To change the calculation zone, click the **Calculation Options** button in the Scanning Analysis dialog.



- Currently, only the "From x: / To x: " method for determining the calculation zone is supported. See Mean, Std Dev, CV for more information.

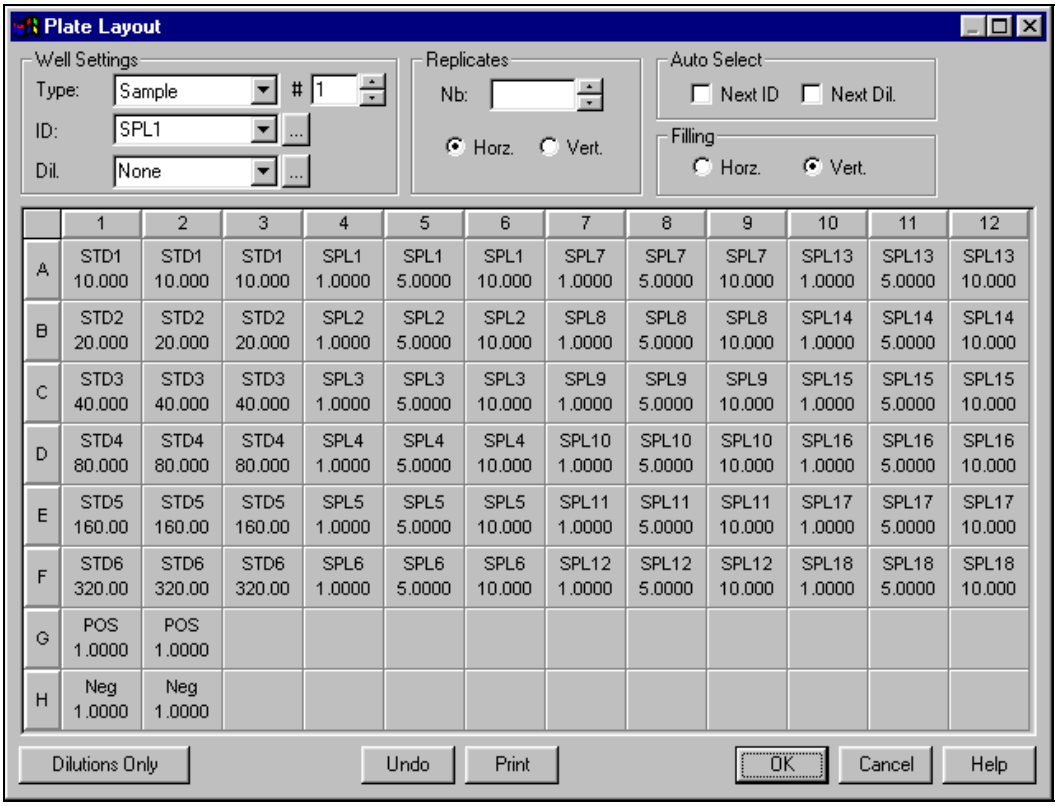
Plate Layout

KC4 allows you to describe the layout of the microplate(s) that will be associated with a protocol. You are not *required* to do this. To perform blank well subtraction, generate curves, assign dilution values, or define formulas that reference well identifiers, however, you must create a **Plate Layout.** The process involves:

- Selecting a well type (Blank, Assay Control, Sample, Sample Control, or Standard).
- Defining well identifier(s) to be associated with the well type.
- Defining dilution or concentration values (if required).
- Assigning the well identifiers to the appropriate locations on a row x column grid.

To perform these tasks, select **Protocol|Plate Layout,** or click the Layout toolbar icon.

The Plate Layout dialog appears, in the row x column format of the **plate type** selected under Protocol|Reading:



	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 10.000	STD1 10.000	STD1 10.000	SPL1 1.0000	SPL1 5.0000	SPL1 10.000	SPL7 1.0000	SPL7 5.0000	SPL7 10.000	SPL13 1.0000	SPL13 5.0000	SPL13 10.000
B	STD2 20.000	STD2 20.000	STD2 20.000	SPL2 1.0000	SPL2 5.0000	SPL2 10.000	SPL8 1.0000	SPL8 5.0000	SPL8 10.000	SPL14 1.0000	SPL14 5.0000	SPL14 10.000
C	STD3 40.000	STD3 40.000	STD3 40.000	SPL3 1.0000	SPL3 5.0000	SPL3 10.000	SPL9 1.0000	SPL9 5.0000	SPL9 10.000	SPL15 1.0000	SPL15 5.0000	SPL15 10.000
D	STD4 80.000	STD4 80.000	STD4 80.000	SPL4 1.0000	SPL4 5.0000	SPL4 10.000	SPL10 1.0000	SPL10 5.0000	SPL10 10.000	SPL16 1.0000	SPL16 5.0000	SPL16 10.000
E	STD5 160.00	STD5 160.00	STD5 160.00	SPL5 1.0000	SPL5 5.0000	SPL5 10.000	SPL11 1.0000	SPL11 5.0000	SPL11 10.000	SPL17 1.0000	SPL17 5.0000	SPL17 10.000
F	STD6 320.00	STD6 320.00	STD6 320.00	SPL6 1.0000	SPL6 5.0000	SPL6 10.000	SPL12 1.0000	SPL12 5.0000	SPL12 10.000	SPL18 1.0000	SPL18 5.0000	SPL18 10.000
G	POS 1.0000	POS 1.0000										
H	Neg 1.0000	Neg 1.0000										

- For readers that support **multiple plate formats,** make sure that the correct plate type is selected in the Reading parameters dialog before creating the plate layout.
- Use the **Well Settings** options to select a well type, customize the text and color of its IDentifier(s), and define dilutions or concentrations (if required).
- Enter the number of **Replicates** then select the replicate mapping direction, **Horizontal** or **Vertical.**
- Check an **Auto Selection** option to automatically increment well ID group numbers and/or concentration or dilution values.
- Select the group mapping direction, **Horizontal** or **Vertical.**
- **Highlight** one or more or wells in the grid to assign the identifier(s).

Tips:

- Click the **Undo** button to undo the last action.
- Click the **Print** button to print the current plate layout. If the plate contains 384 wells, the printout will cover four pages, one per plate quadrant.
- The Plate Layout dialog can be **maximized** to fill your entire screen. Click the maximize icon in the upper right corner of the Plate Layout dialog.
- Within the grid, **rows** and **columns** can be **resized** (to see all wells of a 384-well plate, for example). Place the cursor over a row or column separator until it changes shape. Click and hold the mouse, then drag the cursor up or to the left to make rows or columns smaller. Drag the cursor down or to the right to make rows or columns larger.

Well Types and IDentifiers

Select and customize Well Types and Well IDentifiers within the **Well Settings** area of the Plate Layout dialog, then assign them to one or more locations within the **grid** area of the dialog.

- The **Well Type** tells KC4 which function to perform using the measurement value in the well. KC4 supports five different Well Types:
  - **Blank,** used during full plate blank subtraction.
  - **Assay Control,** used during assay validation or as calibrators.
  - **Sample,** representing the unknown samples to be processed.
  - **Sample Control,** representing the control well(s) that are associated with a specific sample.
  - **Standard,** used to generate a standard curve on which data will be interpolated.
- The **Well ID** is the descriptive link between the measurement value retrieved for the well and the Well Type assigned to it.

  For example: The average of the values in the wells of type Blank and labeled with the Well ID "BLK" will be subtracted from every well on the plate, as a part of KC4's blank subtraction function.

Selecting and Customizing Well Types & IDs

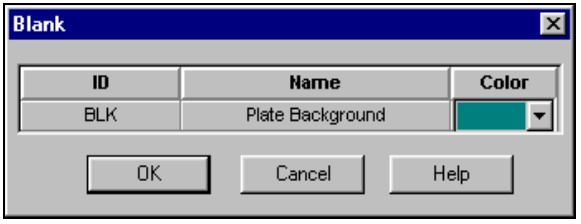
The customization features vary among the different Well Types. Each Well Type is addressed individually starting below and continuing on the following pages.

Blank Wells

During data reduction, the average of the raw data values in the blank wells is subtracted from the raw data values in all wells on the plate (or from the corrected data values if a Blank Plate was read).

KC4 requires that the same identifier be used for all blank wells. The maximum number of blank wells that can be defined is limited only by the number of available wells on the plate. You cannot define concentrations or dilutions for blank wells.

The default blank well ID is BLK, and its default color is white (it will print in black on reports). To change the ID text and/or color, or to define a descriptive name for the blank wells for use on reports, click the "…" box to the right of the ID field.



- Enter an **ID** using 1 to 10 characters.

  The ID can contain letters, numbers, and underscores. It cannot contain any spaces. 'Blank_Well' is valid, 'Blank Well' is not.

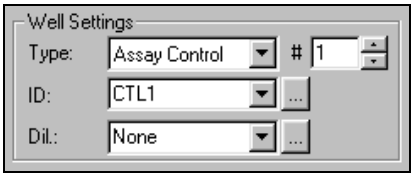
  The ID cannot be identical to a formula operand (X, MIN, MAX, LOG, POW, SQRT, CV, SD, and MEAN are not valid IDs).

  If an ID is currently used in the grid, its text can be **changed** here. The change will automatically be reflected in the grid.

- (Optional) Enter a descriptive **Name** using up to 20 alphanumeric characters. This name can be included on reports.

- Click on the **Color** box to open a drop-down list showing all of the available colors. Click on the desired color.

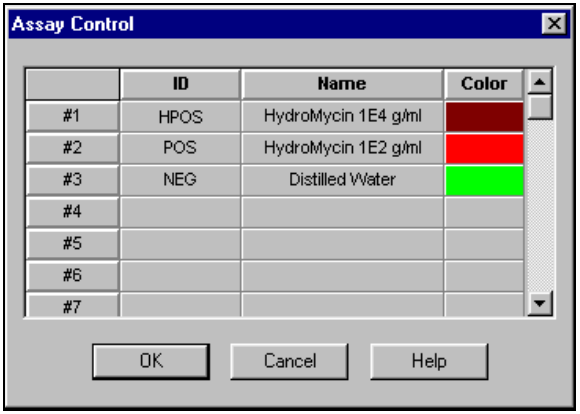
Assay Control Wells



Assay control wells are typically used to validate the assay, or they can be used as calibrators.

Unique IDs must be used to distinguish between the different control groups (e.g. "NC" for negative controls, "PC" for the positive controls). The maximum number of unique assay control well IDs that can be defined is limited only by the number of available wells on the plate. Concentrations and dilutions can be optionally assigned.

The default IDs are CTL1, CTL2, CTL3, etc., each with a different color. To change the IDs and/or colors, or to define descriptive names for use on reports, click the "…" box to the right of the ID field.



- Enter an **ID** using 1 to 10 characters.

  The ID can contain letters, numbers, and underscores. It cannot contain any spaces. 'High_POS' is valid, 'High POS' is not.

  The ID cannot be identical to a formula operand (X, MIN, MAX, LOG, POW, SQRT, CV, SD, and MEAN are not valid IDs).

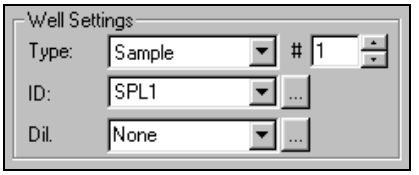
  If an ID is currently used in the grid, its text can be **changed** here. The change will automatically be reflected in the grid.

  If an ID is not currently used in the grid, it can be **deleted** from this list by deleting the contents of the ID field.

- (Optional) Enter a descriptive **Name** using up to 20 alphanumeric characters. This name can be included on reports.

- Click on the **Color** box to open a drop-down list showing all of the available colors. Click on the desired color.

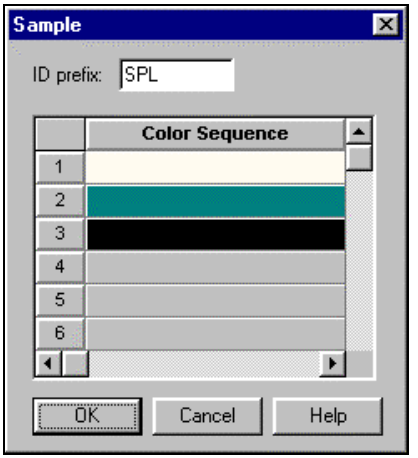
Sample Wells



Sample wells contain the unknown sample solutions to be processed. Dilutions or concentrations can be optionally assigned.

Unique IDs should be used to distinguish between the different sample groups. The maximum number of unique sample well IDs that can be defined is limited only by the number of available wells on the plate.

The default ID prefix is SPL, and the default IDs are SPL1, SPL2, SPL3, etc. The default color for all IDs is black. To change the ID prefix and/or colors, click the "…" box to the right of the ID field.



- Enter an **ID Prefix** using 1 to 7 characters.

  The prefix can contain letters, numbers, and underscores.
  It cannot contain any spaces, and it cannot end in a number.

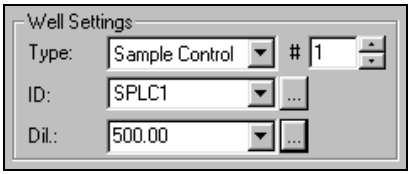
  If the prefix is currently used in the grid, its text can be **changed** here. The change will automatically be reflected in the grid.

- For each group number, click on the **Color Sequence** box to open a drop-down list showing all of the available colors. Click on the desired color.

  KC4 uses the first color for SPL1, then the second for SPL2, and so on. It restarts with the first color when the whole list of colors has been used.

  To remove the last color of the current sequence, set it to **End of Sequence** (the last item in the list).

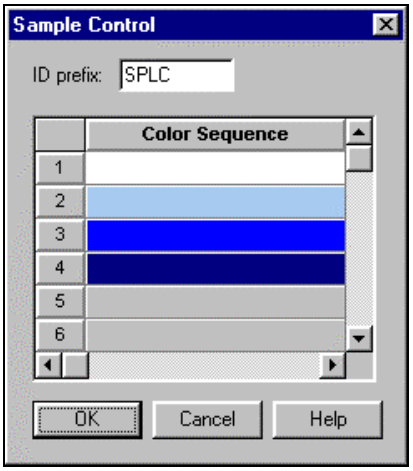
Sample Control Wells



Sample Controls are the controls that are associated with specific samples. For example, SPLC1 is associated with SPL1 (assuming the default names are used). This well type can be used to represent sample blanks, or spikes.

Unique IDs should be used to distinguish between the different sample control groups. The maximum number of unique sample control well IDs that can be defined is limited only by the number of available wells on the plate.

The default ID prefix is SPLC, and the default IDs are SPLC1, SPLC2, SPLC3, etc. The default color for all IDs is dark purple. To change the ID prefix and/or colors, click the "…" box to the right of the ID field.



- Enter an **ID Prefix** using 1 to 7 characters.

  The prefix can contain letters, numbers, and underscores.
  It cannot contain any spaces, and it cannot end in a number.

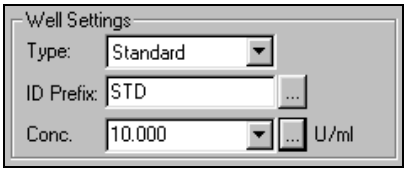
  If the prefix is currently used in the grid, its text can be **changed** here. The change will automatically be reflected in the grid.

- For each group number, click on the **Color Sequence** box to open a drop-down list showing all of the available colors. Click on the desired color.

  KC4 uses the first color for SPLC1, then the second for SPLC2, and so on. It restarts with the first color when the whole list of colors has been used.

  To remove the last color of the current sequence, set it to **End of Sequence** (the last item in the list).

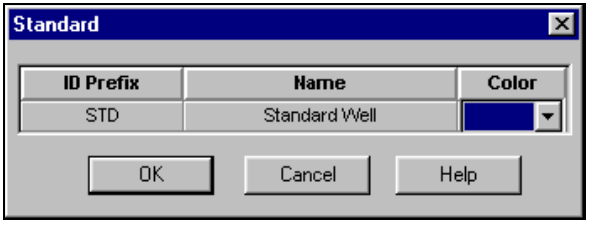
Standard Wells



Standard wells are used to generate a standard curve on which data will be interpolated. Expected concentrations must be defined for each standard group.

Unique IDs must be used to distinguish between the different standard groups. The minimum number of standard groups that can be defined is 2, and the maximum number is 20. **Note:** Each curve fit type has its own required minimum number of standards.

The default ID prefix is STD, and the default IDs are STD1, STD2, STD3, etc. The default color for all standard wells is dark blue. To change the ID prefix and/or color, click the "…" box to the right of the ID field.



- Enter an **ID Prefix** using 1 to 7 characters.

  The prefix can contain letters, numbers, and underscores.
  It cannot contain any spaces, and it cannot end in a number.

  If the prefix is currently used in the grid, its text can be **changed** here. The change will automatically be reflected in the grid.

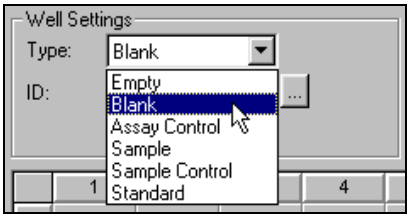
- (Optional) Enter a descriptive **Name** using up to 20 alphanumeric characters. This name can be included on reports.

- Click on the **Color** box to open a drop-down list showing all of the available colors. Click on the desired color.

Assigning Well IDs

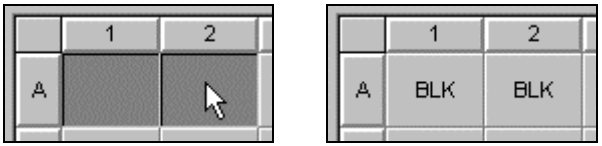
After defining the Well Settings, assign the Well ID(s) in the grid area of the Plate Layout dialog. Here is a very simple example for assigning blank wells to the plate:

- Select the Blank well type from the **Type** drop-down menu:



- The **ID** field shows the default identifier, BLK:

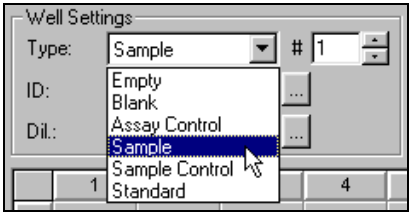
- Assuming you wish to use the "BLK" identifier, **highlight** the well(s) in the template that should contain blanks, then release the mouse button:



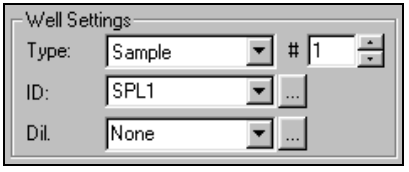
- The average of the measurement values in wells A1 and A2 will be subtracted from every well on the plate.

KC4 provides options for defining replicates and automatically updating the Well ID numbers. The following example shows you how to define **Replicates** and use the **Auto Select** and **Filling** features to assign samples in triplicate.

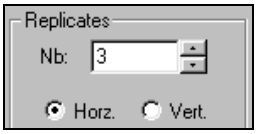
- Select the Sample well type from the **Type** drop-down menu:



- The **ID** field shows the first ID in the sequence, SPL1:



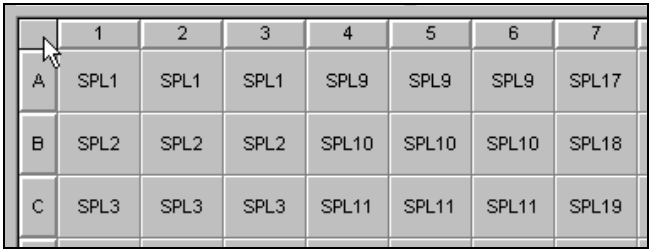
- Set the number of **Replicates** to 3, then select the **Horizontal** filling option. Sample *replicates* will be assigned horizontally, A1/A2/A3, B1/B2/B3, and so on.



- Choose the **Auto Select** option **Next ID.** This automatically increments sample *groups*, SPL1, SPL2, SPL3, and so on.

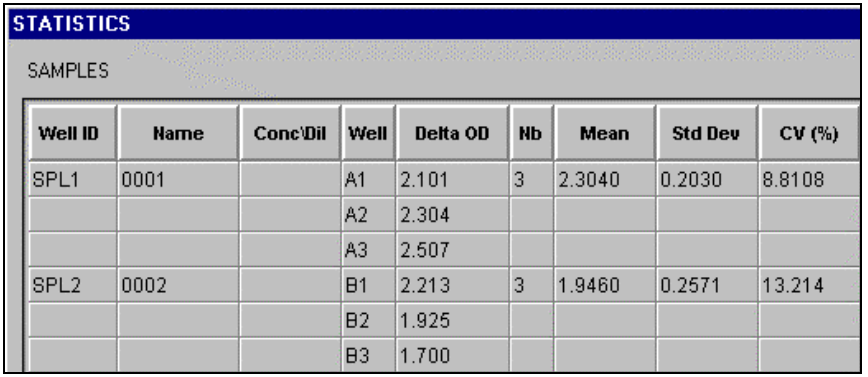
- Select the **Filling** option **Vertical** to assign *groups* vertically.

- Finally, go to the grid and click the mouse once in the box above the A and to the left of the 1. This fills all wells with samples.



	1	2	3	4	5	6	7
A	SPL1	SPL1	SPL1	SPL9	SPL9	SPL9	SPL17
B	SPL2	SPL2	SPL2	SPL10	SPL10	SPL10	SPL18
C	SPL3	SPL3	SPL3	SPL11	SPL11	SPL11	SPL19

- During data reduction, KC4 will automatically calculate and display the Mean, Standard Deviation, and CV % for each sample group (click the **Statistics** button from within the Data display to view this information):



STATISTICS

SAMPLES

Well ID	Name	Conc\Dil	Well	Delta OD	Nb	Mean	Std Dev	CV (%)
SPL1	0001		A1	2.101	3	2.3040	0.2030	8.8108
			A2	2.304				
			A3	2.507				
SPL2	0002		B1	2.213	3	1.9460	0.2571	13.214
			B2	1.925				
			B3	1.700				

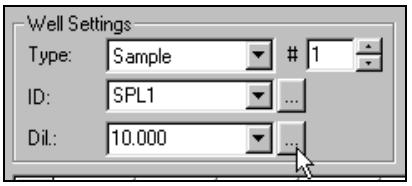
Defining and Assigning Dilutions

KC4 provides the ability to define **dilutions** for Assay Controls, Samples, and Sample Controls.

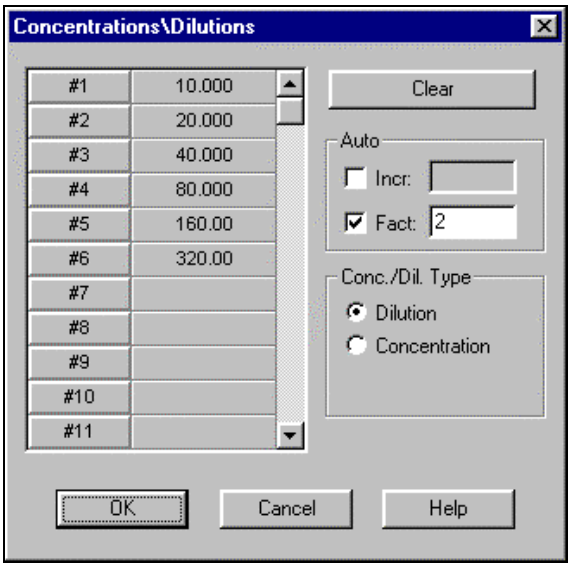
Defining Dilutions

Dilution values can be assigned to wells when the well IDs are assigned, or they can be assigned to existing well IDs by clicking the **Dilutions Only** button. This section discusses assigning dilutions and well IDs at the same time.

When defining dilutions, start by selecting the desired well type. The dilution values will remain associated with this well type. Customize the well IDs if necessary.



When the Type and ID fields are set, click the "…" button to the right of the **Dil.** Field. The **Concentrations\Dilutions** dialog appears:



Use this dialog to enter the dilution values required for the assay. Dilutions are must be entered in ascending or descending order, starting with Dilution **#1** (10.000 in the example above):

- Choose the Conc./Dil. Type option **Dilution.**
- Enter the **starting dilution** in cell #1. Subsequent dilutions can be entered manually or automatically:

  Enter dilutions **manually** in cells #2, #3, and so on. Use the down arrow key to advance to the next cell, or click the mouse in the desired cell.

  To increase dilutions automatically, choose an **Auto** option of **Incr** or **Fact.** Enter the required Increment or Factor value, then click on cell #2 to assign the next dilution. Use the down arrow key to advance to the next cell, or click the mouse in the desired cell.

Incr: Increments dilutions by the entered value. In the example above, the starting dilution is 10, and subsequent dilutions increase by 10. Enter a negative number (e.g. -10) to decrement dilution values.

Fact: Increase dilutions by an entered factor value. If the starting dilution is 2, increasing by a factor of 2, subsequent dilutions become 4, 8, 16, 32, 64, and so on.

- Click the **Clear** button to delete all values from all cells.
- When all of the dilution values have been entered, click **OK** to return to the Plate Layout dialog.

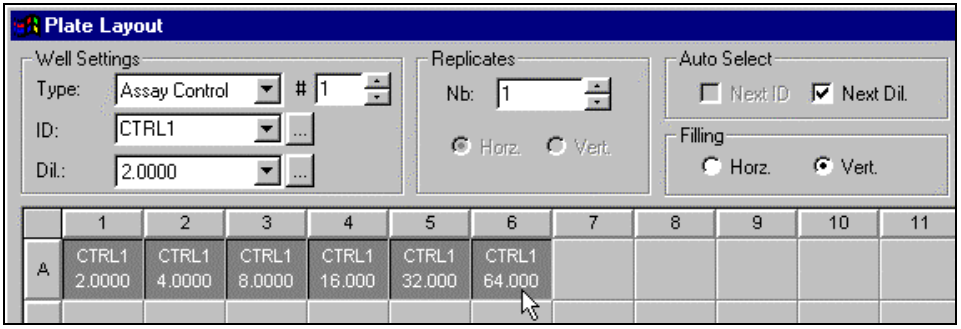
Assigning Dilutions

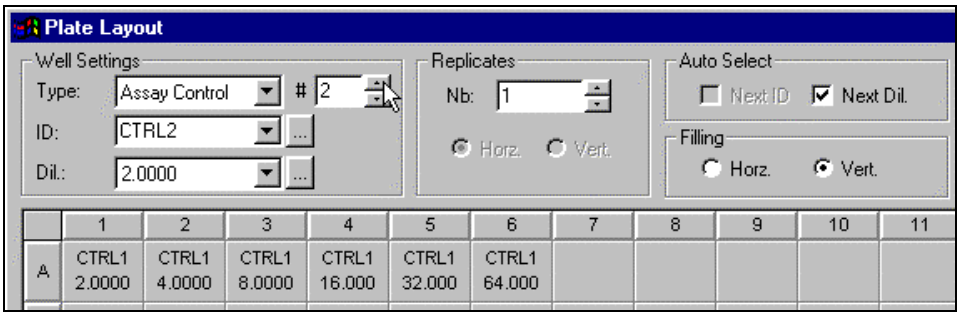
There are different techniques for assigning dilutions. You may wish to experiment with the software, to get comfortable with assigning Replicates, choosing neither, one, or both Auto Select options, and toggling the Horizontal/Vertical filling options. It might also be helpful to sketch your plate map on a piece of paper to use as a reference.

To assign dilutions (with Well IDs):

- Select the necessary **Well Settings** (Type, #, and ID), then choose the first dilution value to be assigned.
- Enter the number of dilution **Replicates** per control or sample.
- Choose the dilution replicate filling direction, **Horizontal** or **Vertical.**
- To advance well IDs and/or dilution values automatically, choose **Next ID** (if available), and/or **Next Dil.**
- Choose the dilution group filling direction, **Horizontal** or **Vertical.**
- **Highlight** the well(s) to assign dilutions and well IDs.

Examples are provided on the following pages.





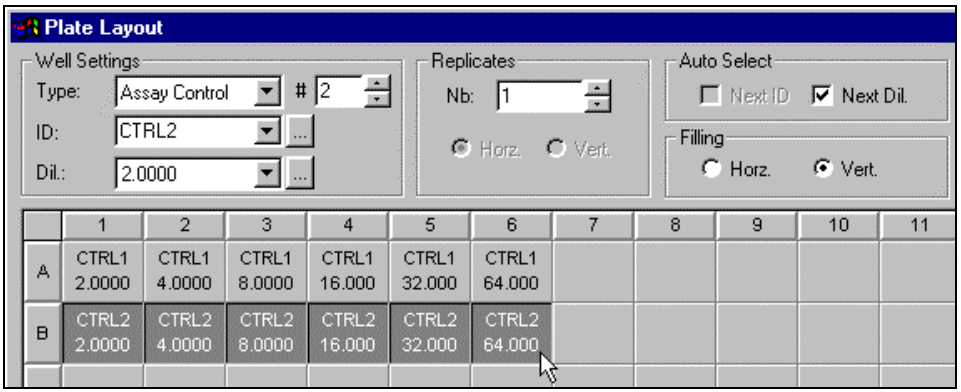
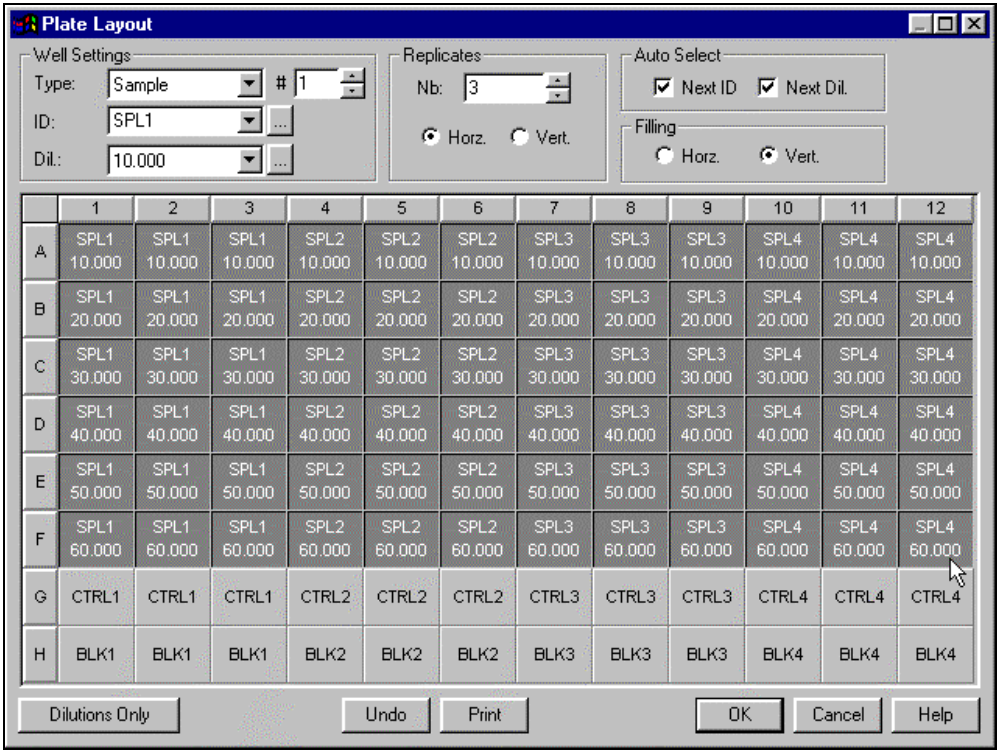


Plate Layout
Well Settings
Type: Assay Control # 2
ID: CTRL2
Dil.: 2.0000
Replicates
Nb: 1
Horz. Vert.
Auto Select
Next ID Next Dil.
Filling
Horz. Vert.
1 2 3 4 5 6 7 8 9 10 11
A CTRL1 2.0000 CTRL1 4.0000 CTRL1 8.0000 CTRL1 16.000 CTRL1 32.000 CTRL1 64.000
B CTRL2 2.0000 CTRL2 4.0000 CTRL2 8.0000 CTRL2 16.000 CTRL2 32.000 CTRL2 64.000

- Dilution values are defined as 2.000, 4.000, 8.000, 16.000, 32.000, and 64.000.
- The Auto Select option Next Dilution is selected. The Next ID option is not available for Assay Control groups; they must be incremented manually.
- Wells A1 through A6 are highlighted to assign CTRL1 and its dilutions. CTRL2 is then selected and assigned to wells B1 through B6.



- Dilution values are defined as 10.000, 20.000, 30.000, 40.000, 50.000, and 60.000.
- Replicates is set to 3, and both Auto Select options are enabled.
- Wells A1 through F12 are highlighted to assign IDs and dilutions.

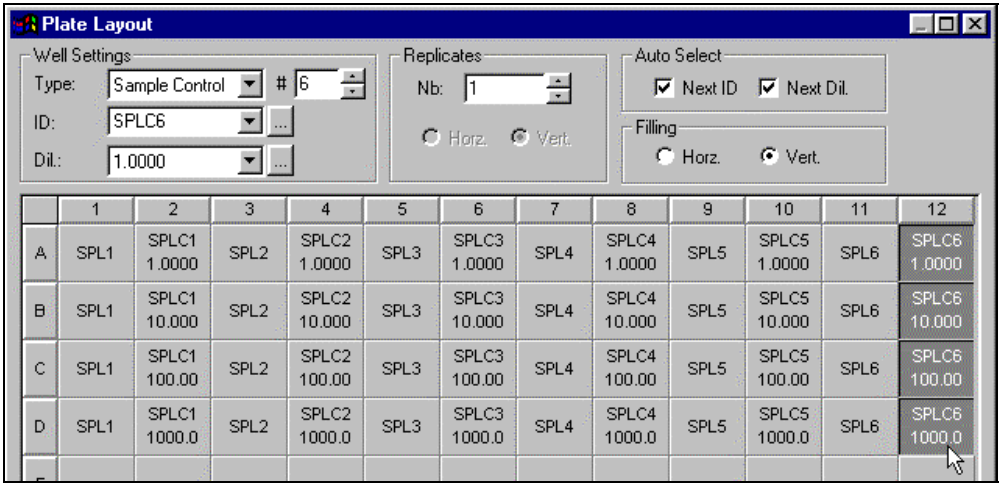


Plate Layout
Well Settings
Type: Sample Control
6
ID: SPLC6
Dil.: 1.0000
Replicates
Nb: 1
Horz. Vert.
Auto Select
Next ID Next Dil.
Filling
Horz. Vert.
1 2 3 4 5 6 7 8 9 10 11 12
A SPL1 SPLC1 1.0000 SPL2 SPLC2 1.0000 SPL3 SPLC3 1.0000 SPL4 SPLC4 1.0000 SPL5 SPLC5 1.0000 SPL6 SPLC6 1.0000
B SPL1 SPLC1 10.000 SPL2 SPLC2 10.000 SPL3 SPLC3 10.000 SPL4 SPLC4 10.000 SPL5 SPLC5 10.000 SPL6 SPLC6 10.000
C SPL1 SPLC1 100.00 SPL2 SPLC2 100.00 SPL3 SPLC3 100.00 SPL4 SPLC4 100.00 SPL5 SPLC5 100.00 SPL6 SPLC6 100.00
D SPL1 SPLC1 1000.0 SPL2 SPLC2 1000.0 SPL3 SPLC3 1000.0 SPL4 SPLC4 1000.0 SPL5 SPLC5 1000.0 SPL6 SPLC6 1000.0

- Dilution values are defined as 1.000, 10.000, 100.000, and 1000.000.
- Replicates is set to 1, and both Auto Select options are selected.
- Wells A2 through D2 are highlighted to assign the ID and dilutions, then A4 through D4, and so on to A12 through D12.

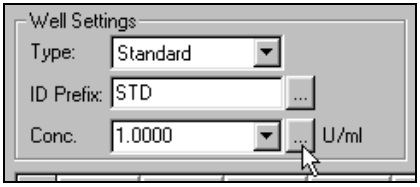
Defining and Assigning Concentrations

If the plate layout contains Standard wells, or if you want to generate curves based on control or sample wells (e.g. for a titer assay), the location and the expected **concentrations** of these wells must be defined and assigned in the plate layout. KC4 uses the expected concentration values as the basis for calculating the curve; they represent the x-axis values. The measurement values retrieved from the reader (or the results of transformations applied to them), represent the y-axis values.

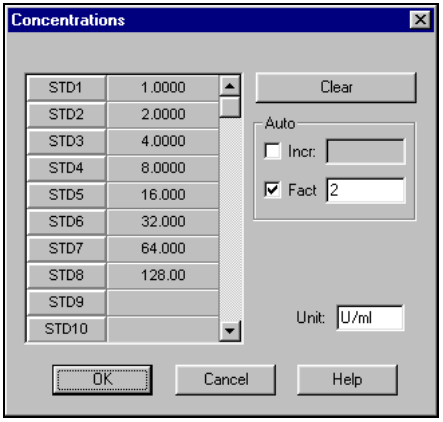
KC4 provides the ability to define concentrations for Assay Controls, Samples, Sample Controls, and Standards.

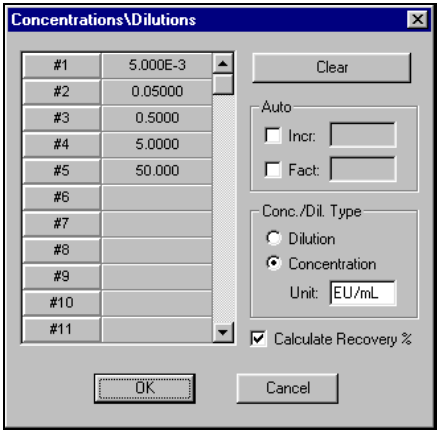
Defining Concentrations

When defining concentrations, start by selecting the desired well type. The concentration values will remain associated with this well type. Customize the well IDs if necessary.



When the Type and ID fields are set, click the "…" button to the right of the **Conc.** field (for well types other than Standard, this field will initially be labeled “Dil.”). The **Concentrations** or **Concentrations\Dilutions** dialog will appear, depending on the well type.





Use this dialog to enter the expected concentration values required for the assay. Values must be entered in ascending or descending order, starting with concentration **#1** (1.000 and 5.000E-3 in the examples above):

- Enter the **starting concentration** in cell #1. Subsequent concentrations can be entered manually or automatically:

  When entering concentrations manually, use the down arrow key to advance to the next cell, or click the mouse in the desired cell.

To increase concentrations automatically *after* entering the starting concentration, choose an **Auto** option of **Incr** or **Fact** then define the required Increment or Factor value. Click on cell #2 to assign the next concentration. Use the down arrow key to advance to the next cell, or click the mouse in the desired cell.

Incr: Increments concentrations by the defined value. If the starting concentration is 10.000 with an Increment of 10, subsequent concentrations become 20.000, 30.000, and so on. Enter a negative number (e.g. -5) to decrement concentration values.

Fact: Increases concentrations by the defined value. If the starting concentration is 1.000 with a Factor of 2, subsequent concentrations become 2.000, 4.000, 8.000, 16.000, and so on.

- (Optional) Enter the **Unit** of measure for the X-axis values on the curve, using up to five alphanumeric characters.
- Click the **Clear** button to delete all values from all cells.
- For Sample Controls, KC4 provides the **Calculate Recovery %** option. If this feature is enabled, KC4 automatically generates a "Recovery %" results data set, containing the results of the following formula:

  [Conc(SPLC*n*) - Conc(SPL*n*) ÷ ExpectedConc(SPLC*n*)] x 100

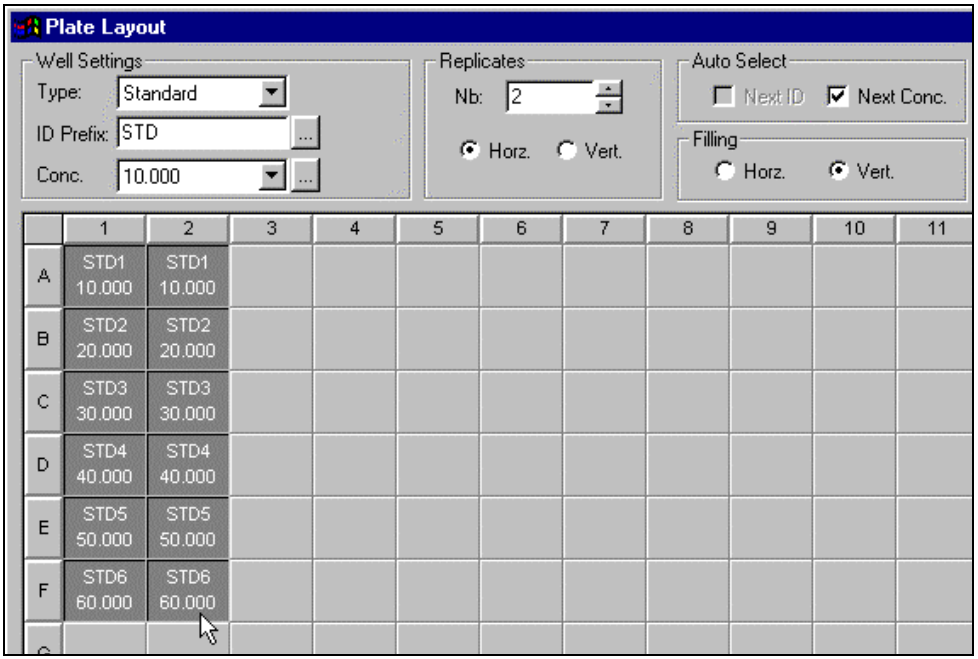
- When all of the concentration values have been entered, click **OK** to return to the Plate Layout dialog.

Assigning Concentrations

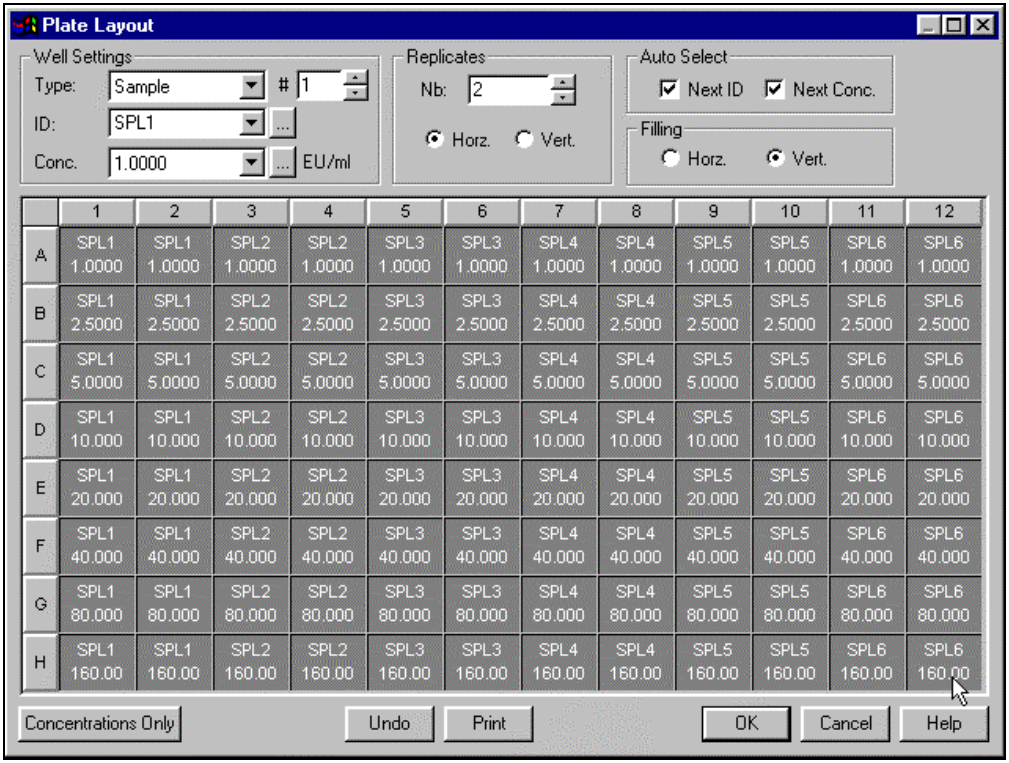
To assign concentrations (with well IDs):

- Select the necessary **Well Settings** (Type, #, and ID), then choose the first concentration value to be assigned.
- Enter the number of standard, sample, or control **Replicates.**
- Choose the *replicate* filling direction, **Horizontal** or **Vertical.**
- If you want well IDs and/or concentration values to advance automatically, choose **Next ID** (if available), and/or **Next Dil.**
- Choose the *group* filling direction, **Horizontal** or **Vertical.**
- **Highlight** the well(s) to assign the concentrations and well IDs.

Examples are provided below and on the next page.

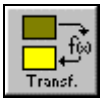


- Concentrations are defined as 10, 20, 30, 40, 50, and 60.
- The Auto Select option Next Concentration is selected. The Next ID option is not available for Standard groups; they must be incremented manually.
- Wells A1 through F2 are highlighted to assign the concentrations and IDs.



- Concentrations are defined as 1, 2.5, 5, 10, 20, 40, 80, and 160.
- Replicates is set to 2, and both Auto Select options are selected.
- Wells A1 through H12 are highlighted to assign the concentrations and IDs.

Single Plate Transformations



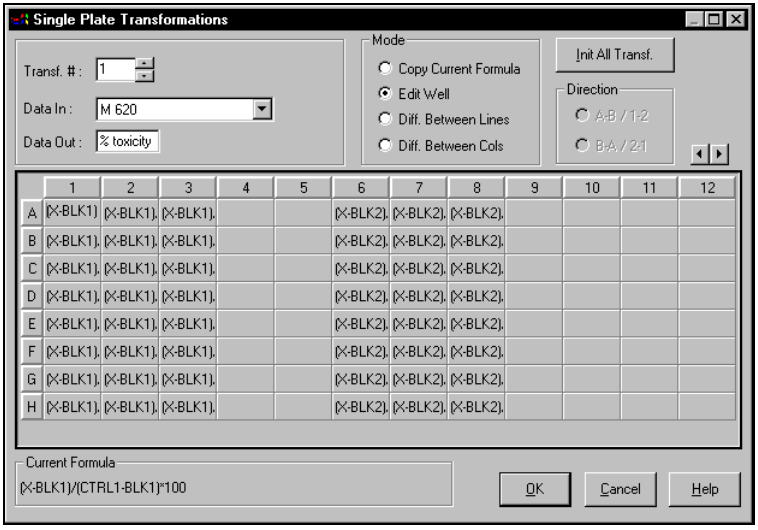
KC4 provides for up to three levels of **Single Plate Transformations.** The three different levels of transformations may be interdependent, or completely independent of each other. Typical uses include:

- Using specific wells as local blanks for other wells.
- Calculating ratios between wells.

The **Single Plate Transformation** dialog functions similarly to a spreadsheet. The data entry template resembles a microplate in row x column format. Formulas are entered in "cells" representing the wells within the microplate.

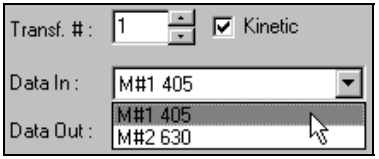
To define **Single Plate Transformations:**

- For readers that support multiple plate formats, ensure the correct format is selected under **Protocol|Reading.**
- Ensure that identifiers have been assigned under **Protocol|Plate Layout.**
- Select **Protocol|Single Plate Transformations:**


Single Plate Transformations
Transf. # : 1
Data In : M 620
Data Out : % toxicity
Mode
Copy Current Formula
Edit Well
Diff. Between Lines
Diff. Between Cols
Init All Transf.
Direction
A-B / 1-2
B-A / 2-1
1 2 3 4 5 6 7 8 9 10 11 12
A (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
B (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
C (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
D (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
E (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
F (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
G (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
H (X-BLK1), (X-BLK1), (X-BLK1), (X-BLK2), (X-BLK2), (X-BLK2),
Current Formula
(X-BLK1)/(CTRL1-BLK1)*100
OK
Cancel
Help

  - Place the cursor in a well and enter the desired formula. The formula is visible in the **Current Formula** box at the bottom of the dialog.
  - The **Transf. #** field indicates the current transformation level. Level 1 transformations can be passed for further manipulation into Level 2 and Level 3 transforms for successive manipulation.
  - **Data In** represents the data set upon which the formulas will be applied. The **Data In** choices will vary depending on the defined protocol parameters.

- For kinetic protocols, a **Kinetic** checkbox is provided. If this box is checked, the Data-In options represent plate data, such as "M#1 405" or "M#2 630". If this box is not checked, the Data-In options represent the results of kinetic calculations, such as "Max V" and "Coeff Reg".



- The **Data Out** field assigns a name to the transformed data. The transformed data can then be used for further calculations, including Multi Plate Transformations.
- Select **Copy Current Formula** to copy the current formula into one or more wells. Clicking on the title row or column auto-fills the wells selected with the current formula. Clicking on the top left corner auto-fills the whole plate.
- Select **Edit Well** to edit the formula in a particular well.
- Select **Diff. Between Lines** to perform subtraction between rows, then select **A-B** or **B-A.**
- Select **Diff. Between Cols** to perform subtraction between columns, then select **1-2** or **2-1.**
- Click **Init. All Transf.** to reinitialize all Single Plate Transformations to their most recently saved settings.
- Click to **expand** the width of the columns, to view and access long formulas. The maximum expansion shows a single column of wells in full screen.
- Click to **contract** the width of the columns.

Formula Syntax

The following tables contain **symbols** and **functions** that can be used in Single-Plate Transformations:

Type of Symbol	Description/Example
Algebraic symbols	+ , - , / , * , ( )
Figures (scientific notation)	2.45E-08
x or X	Represents the current well value.
coordinates of the well	Represents the value of a particular well, such as A2 or H12.

Functions Applied to the Identifier	Description/Example
MEAN[POS1]	Represents the mean of identifier POS1. 'POS1' by itself also represents the mean of all POS1 wells on the plate.
SD[NEG1]	Represents the standard deviation of all valid wells in group NEG1.
CV[BLK]	Represents the coefficient of variation of all valid wells in group BLK.

Advanced Mathematical Functions	Description/Example
LOG(x)	Represents the log10 function.
POW(x;y)	Raises x to the power of y.
POW(10;x)	Calculates the Anti-Log of the current well.
SQRT(x)	Square root of x.
MIN(x1;x2;x3...)	Minimum value of defined variables. Example: MIN(A1;B1;C1).
MAX(x1;x2;x3...)	Maximum value of defined variables. Example: MAX(CV[CTRL1];CV[CTRL2]).

Formula Examples

See also the installed sample protocol files for both Single and Multi Plate Transformation examples; these can be found in the SAMPLES folder in your main KC4 folder.

CTRL1-3*SD[CTRL1]

The result of this formula is the mean of the wells containing the identifier CTRL1 minus three Standard Deviations of these wells. It is the equivalent of MEAN[CTRL1]-3*SD[CTRL1].

X+(A5+A6)/2

The result of this formula is the sum of the current well value and the mean of the A5 and A6 wells.

LOG(X/MEAN[POS1])

The result of this formula will log the ratio of the current well over the mean of POS1.

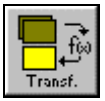
MAX(A1;A2;A3)-MIN(B1;B2;B3)

This formula calculates the difference between the maximum value of A1, A2, A3 and minimum value of B1, B2, B3.

% = (SPLn-BLKn) / (CTRLn-BLKn)

This formula can be used for toxicology assays, to calculate toxicity percentages.

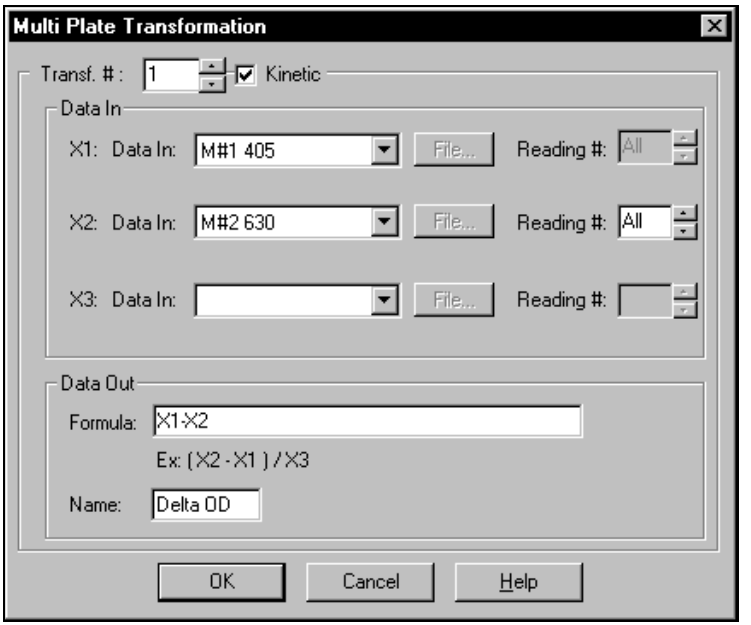
Multi Plate Transformations



KC4 provides for up to three levels of **Multi Plate Transformations.** The three different levels of transformations may be interdependent, or completely independent of each other. In addition, the results of any **Single Plate Transformations** can be incorporated into a Multi Plate Transformation.

To define **Multi Plate Transformations:**

- Ensure that all protocol parameters that have an effect on data reduction have been defined.
- Select **Protocol|Multi Plate Transformations:**



- The **Transf. #** field indicates the current transformation level. Level 1 transformations can be passed for further manipulation into Level 2 and Level 3 transforms for successive manipulation.
- **Data In** represents the data set upon which the formulas will be applied. The **Data In** choices will vary depending on the protocol type and reading parameters.
- Within one Multi Plate Transformation, you can select up to **three** discrete assay data sets for combined calculations. You must select and position each data set in the appropriate location holder (i.e., X1, X2 and X3). The data sets must be of similar type.

- You can select previously stored **data files** (these files must be correctly formatted text files and only contain end-point readings).
- For kinetic protocols, **Reading #** can be set to All, or from 1 to the total number of readings.
- Use the location variables **X1, X2** and **X3** to construct the **Formula.**
- The **Name** field assigns a name to the transformed data and saves the settings and formula. The transformed data can then be used as the Data-In for further calculations, including **Kinetic Analysis** and other **Multi Plate Transformations.**

Syntax

The following table contains **symbols** that can be used in Multi Plate Transformations:

Type of Symbol	**Description/Example**
Algebraic symbols	+ , - , / , * , ( )
Figures (scientific notation)	2.45E-08

Automatic Formula Generation

For readers with multiple wavelength (> 2) reading capability, a default **Multi Plate Transformation** is automatically created when exactly 2 wavelengths or filter sets are specified. These default parameters can be changed.

- The Protocol menu switches to **Advanced Mode.**
- The **Multi Plate Transformation** dialog contains:

  X1 = <Meas. OD#1>

  X2 = <Meas. OD#2>

  Formula = X1 - X2

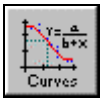
  Data Out = Delta OD
- In addition, if the protocol is **Kinetic:**

  The **'Kinetic'** checkbox becomes enabled.

  The **Reading #** fields for both X1 and X2 are set to **All.**

Note: Once the **Reading Parameters** dialog is saved and closed with 2 filter sets/wavelengths specified, the Delta OD Multi-Plate Transformation will not automatically 'clear' if the protocol is then changed to 1 or 3-6 filter sets/wavelengths. The user must open the Multi Plate Transformation dialog and manually empty/modify the fields.

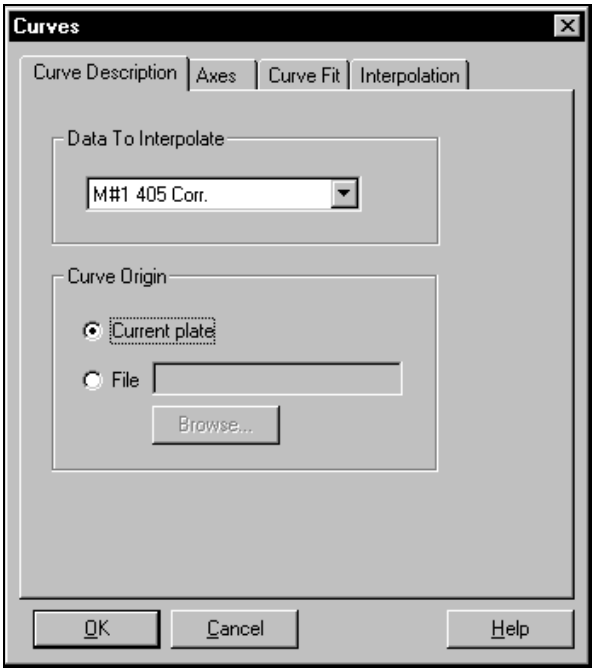
Curves



Select **Protocol|Curves** to define various curve fitting parameters, including the data set to interpolate and the fitting method. Interpolations are defined here as well.

Curve Description

Select **Protocol|Curves** to select the data set to be used for curve calculations, and to specify the origin of the curve.



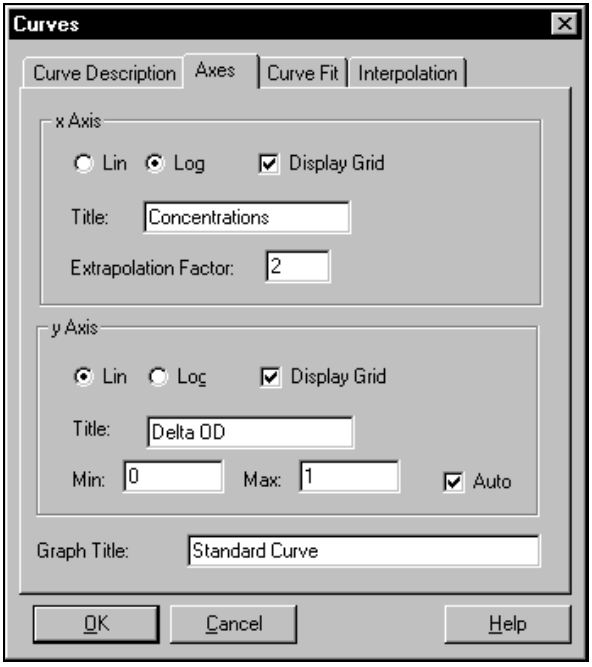
- The **Data to Interpolate** setting represents the type of data to be used for curve fitting.
- Select **Current Plate** if the standard curve will be generated using data from the current plate.
- Select **File** if the standard curve will be based on a previously stored curve. The stored curve will be used as the basis for calculating concentrations for the current plate.

  Click **Browse...** to search for the desired file. The file must have a *.GPH extension. Refer to the **Biograph** module for information on storing curves.

  If a stored curve is used and the plate layout contains standard identifiers, then any use of STD*n* in formulas will refer to the standard well(s) on the current plate and not to the standards in the stored curve.

Curve Axes

By default, the x- and y-Axis data is transformed linearly, and the Extrapolation Factor is set to 1.1. These options can be modified by selecting **Protocol|Curves|Axes.**



- Select either **Lin** or **Log** for the transformation of **x axis** or **y axis** data.
- If **Display Grid** is checked, the graph will be displayed and printed with gridlines.
- The text in the x Axis and y Axis **Title** fields appears on standard curve displays and reports. To **modify** a title (e.g., "% toxicity" or "Delta OD"), overwrite the existing text.
- The **Extrapolation Factor** enables you to extend the abscissa range of the curve fitting routine beyond the limits of the standard data points generated from the assay. See Defining Extrapolation below for more information.

  Exercise caution when using extrapolation, as the implied assumption is that the relationship of the x and y variables is valid outside the range defined by the standards. This type of extrapolation should only be applied when prior knowledge of the relationship is known. Typically this feature is used in assays where linear relationships are known. *Inappropriate use of the Extrapolation Factor feature may invalidate results generated from data falling into the extrapolation zone.*
- By default, the **Min** and **Max y axis** values (for display and reporting purposes) are determined automatically by KC4. These values can be overridden by deselecting Auto and manually entering the Min and Max values.

Defining Extrapolation

The extrapolation factor range must be between **1** and **3.**

Using a factor of **1** implies that the defined upper and lower standards are the limits for concentration interpretation. However, given some assays, the upper and lower limits may be extended, in this case tentative extension of the range may be imposed.

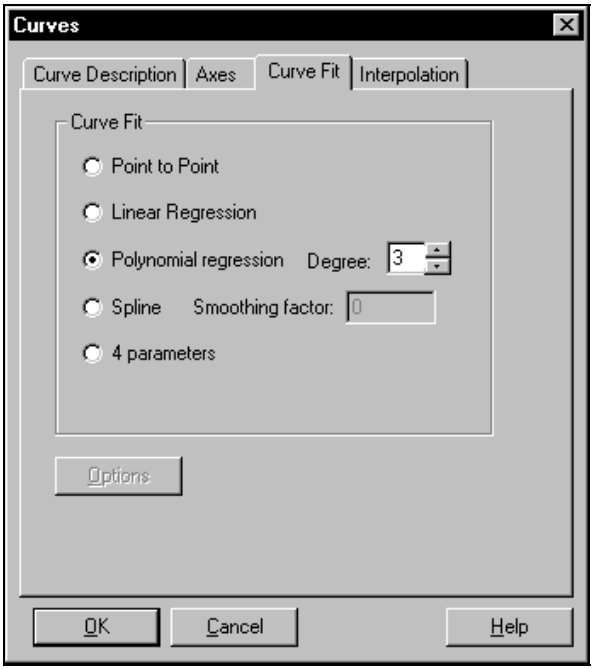
- **Example:** Assuming a linear x axis we wish to extend the upper and lower limit by 20%. The lower limit denoted by the first standard is 50 units. The upper limit denoted by the last standard is 150 units. The dynamic range therefore is 150-50 = 100 units. Imposing the 20% extension means that the new range will be: 100 x 1.2 equals 120 units.

  This represents an increase in the dynamic of 10 units at the lower limit and 10 units at the upper limit. Therefore the new lower limit will be 50-10 equals 40 units. The new upper limit will be 150 + 10 equals 160 units.

- **Notes:** Sample values that fall into the new limits will be calculated. Those that fall outside these limits will be assigned > than or < than, respectively. Extrapolation cannot be used for the Spline routine nor the Point-to-Point fit. These algorithms cannot calculate values outside the upper and lower defined standard points.

Curve Fit

Use the **Protocol|Curves|Curve Fit** dialog to select the method by which the standard curve will be generated. The default setting is **Linear Regression.**



It is important that the appropriate curve fit is applied to the data otherwise the results may be invalid. Whichever curve fitting routine is used, it is essential to check the shape of the curve prior to evaluating data.

The curve fit methods are described below.

Point to Point

Linear interpolation between standard points. The minimum number of standards is 2.

Linear Regression

Linear regression $y = ax + b$.

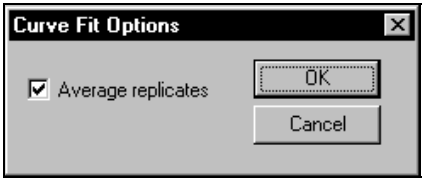
y represents unknown response

x represents concentration

a represents the slope of the linear regression

b represents the intercept to the y axis

- The linear regression fit uses the least squares technique. The better the quality of the fit the more the absolute value of R tends to 1. This type of curve fitting technique can be used when you think the data will fall in a predictable linear pattern. It may be necessary to transform the x and/or y components using Log axes.
- Click the **Options** button to enable or disable the averaging of standard replicates before curve calculation. By default, average replicates is enabled.



Polynomial Regression

This type of regression fitting is an extension of the linear regression equations.

$y = a_n X^n + a_{n-1} X^{n-1} + \ldots a_1 X + a_0$

n represents the degree of the polynomial regression.

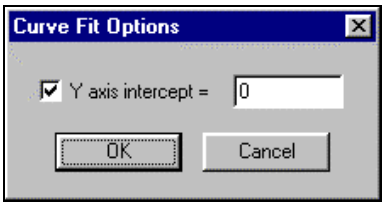
Note: The degree must be less than or equal to the number of standards minus 2. The unknown concentrations are calculated by using an approximate calculation method, linear interpolation between 200 points evenly spaced on the x axis.

- Certain assays require forcing the **curve through the origin.** KC4 provides a feature that allows you to specify the point at which the curve should meet the Y axis.

  Click the **Options** button to enable this feature and then enter the Y axis intercept value. Enter 0 (the default value) to force the curve through the origin.

The value entered here determines the coefficient 'a' of the polynomial equation:

$y = g*x^6 + f*x^5 + e*x^4 + d*x^3 + c*x^2 + b*x + a$



Note: By forcing the curve through the origin, the R-squared value may be decreased because the equation may not describe the data of the standards as well. It is the user's responsibility to check the validity of the data when using this feature.

Spline with Smoothing

Spline fitting with a smoothing factor. The curve will appear smoother as the smoothing factor is increased. The zero factor will force the curve fit through all data points. The minimal number of standards for the spline fit is 4.

Note: The unknown concentrations are calculated using an approximate calculation method, linear interpolation between 200 points evenly spaced on the x axis.

4 Parameter logistic fit

$$y = \frac{a - d}{(1 + (x / c)^b)} + d$$

a = (theoretical) response at concentration = 0

b = measure of slope of curve at its inflection point

c = value of x at inflection point

d = (theoretical) response at infinite concentration

x = concentration

y = response (OD)

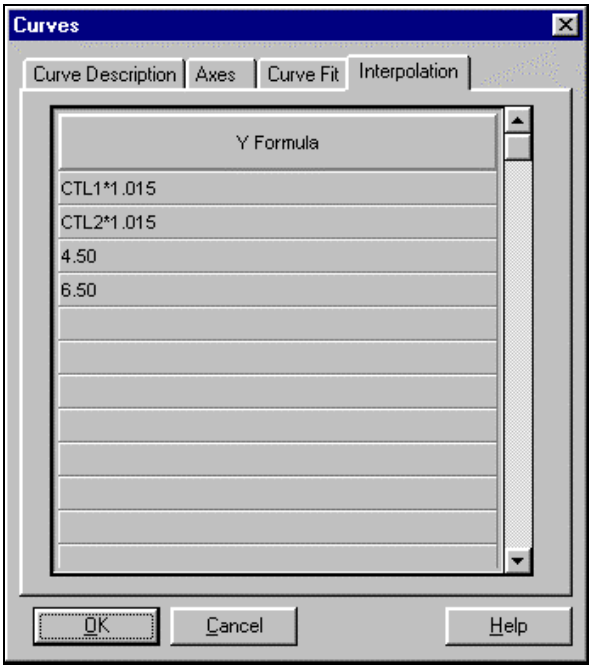
The minimum number of standards is 4.

It is recommended that:

- at least one standard is not far from each asymptote.
- at least 2 standards fall within the linear area of the curve, one either side of the inflection point.

Curve Interpolation

Select **Protocol|Curves|Curve Interpolation** to define Y values and/or formulas for which a concentration will be calculated.



KC4 supports the definition of up to 25 Interpolation values and formulas.

- *Before* defining interpolations that reference **well identifiers,** ensure that all identifiers have been assigned under **Protocol|Plate Layout.**
- Each interpolation can contain any of the following:

  Numeric values, such as `1.500`, `85000`, or `2.0E-8`.

  Well identifiers, such as `NC`, `BLK`, or `STD1`.

  Well coordinates, such as `A1` , `H12`, or `P24`.

  A **formula** combining any or all of the above, such as `CTL1/CTL2*100`.
- Click the **Biograph** button after a plate is read to view the Interpolation Results.
- Interpolation results can be included on **reports** and in **export files.**
- Interpolation formula syntax is described on the next page.

Interpolation Formulas

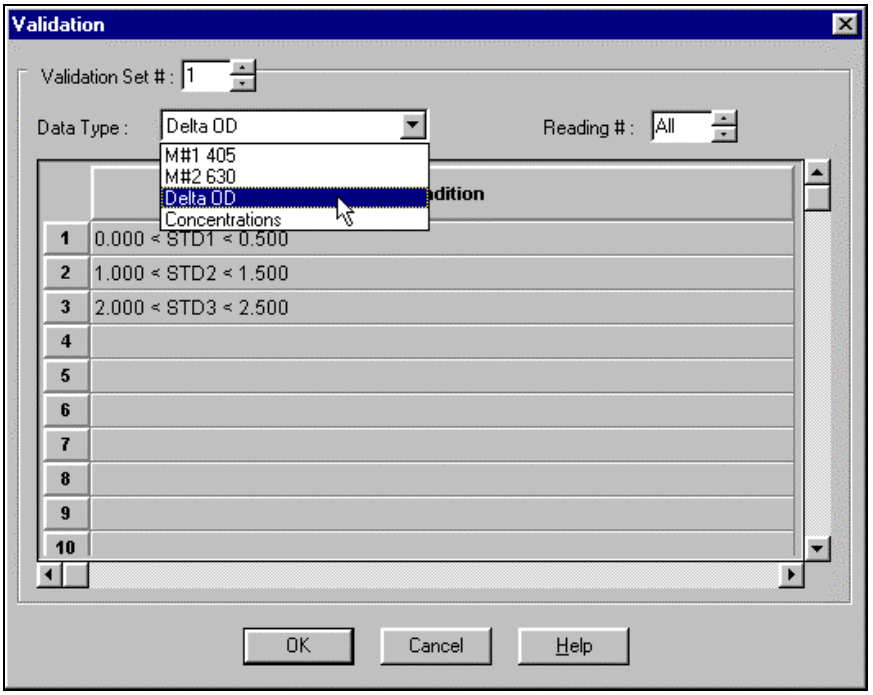
The following table contains valid symbols and functions for use within Interpolation formulas:

Symbol or Function	Description	Example
+	Addition	CTL1+0.100
-	Subtraction or negation	BLK-0.010
/	Division	CTL1/CTL2
*	Multiplication	STD1*0.10
( )	Represents inclusion	(CTL1/CTL2)*100
Well coordinates	The value of a specific well	A1/B1
MEAN[<ID>]	The mean of the specified well identifier	MEAN[POS1]
SD[<ID>]	The standard deviation of the specified well identifier	SD[BLK]
CV[<ID>]	The coefficient of variation of the specified well identifier	CV[STD1]
LOG(<x>)	Represents the LOG10 function	LOG(SPL10)
POW(<x>;<y>)	The value of <x> raised to the power of <y>	POW(STD1;3)
SQRT(<x>)	The square root of <x>	SQRT(A1*B1)
MIN(<x>;<y>;<z>;…)	The minimum of the defined variables	MIN(CV[CTL1]; CV[CTL2])
MAX(<x>;<y>;<z>;…)	The maximum of the defined variables	MAX(A1;B1;C1)

Validation

Validation is a set of determinant criteria used to assess the suitability of calibrators for the inclusion in subsequent calculations. Validation criteria, or **Conditions,** are expressions based on threshold limits previously determined from known standardized conditions.

Select **Protocol|Validation** (with the Protocol Menu in 'Advanced' mode) to define Validation Sets and Conditions.



- Before defining validation criteria, ensure that all identifiers have been selected and defined under **Protocol|Plate Layout.**
- Define up to 5 different **Validation Sets,** each containing up to 10 **Conditions.**
- The **Data Type** setting represents the set of data to which the Conditions equations will be applied.
- The **Conditions** fields allow fixed thresholds and/or algebraic formulas to be used in validating data to satisfy known conditions of the defined assay. A Condition typically combines well identifiers (see **Plate Layout)** with the following symbols:

  < less than

  <= less than or equal to

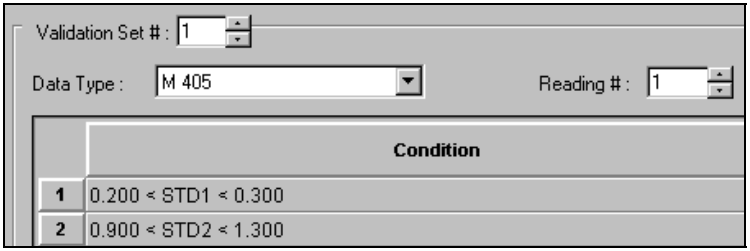
  > greater than

  >= greater than or equal to

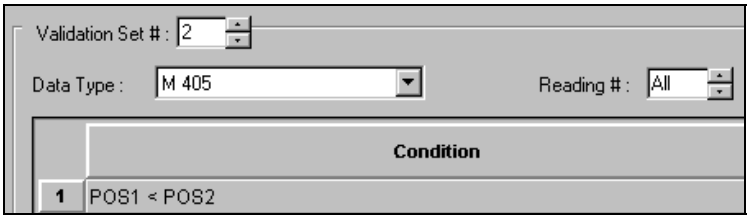
- **Example 1:**

  Conditions to be met:

  1) During the first of several kinetic readings, the mean value of STD1 must fall between 0.200 and 0.300 OD, and the mean value of STD2 must fall between, 0.900 and 1.300 OD.



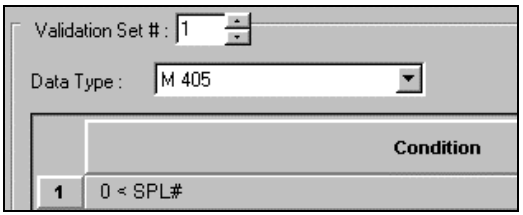
  2) For each kinetic reading point, the mean value of POS1 must be less than the mean value of POS2.



- **Example 2:**

  Condition to be met:

  The mean of every sample replicate group must be greater than 0:

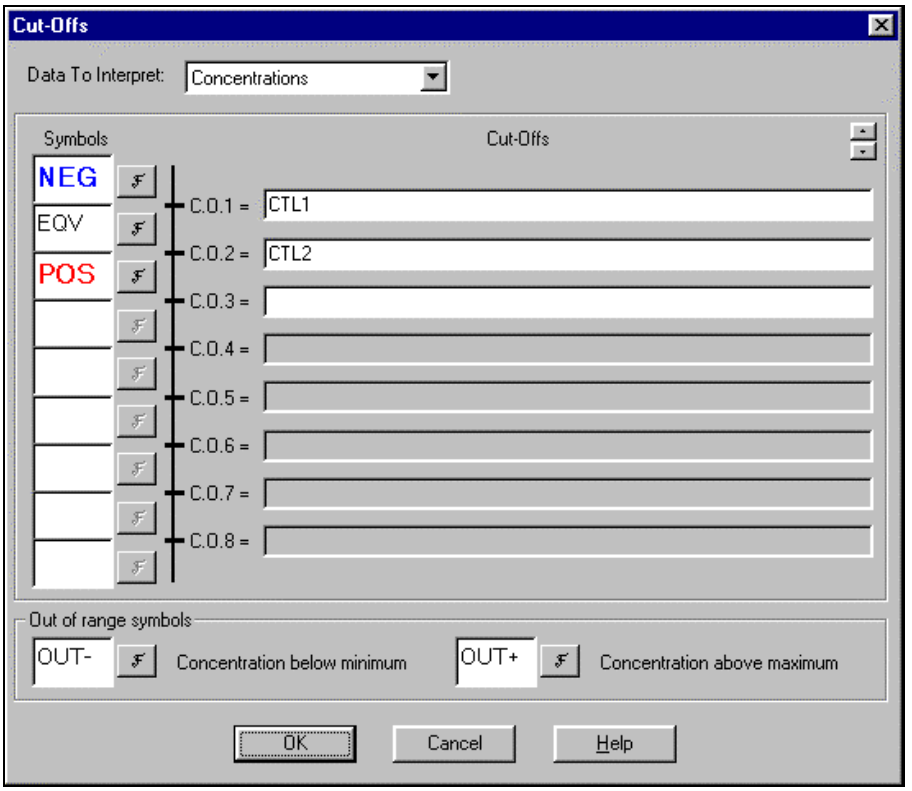


  The # (pound) sign is a generic symbol that can be used to represent all indices of a particular identifier.

Cutoffs

Cutoffs screen selected data into defined categories based on fixed or variable threshold limits. During data reduction, KC4 evaluates results against the cutoff criteria assigns each well a **Symbol.**

Select **Protocol|Cutoffs** (with the Protocol Menu in 'Advanced' mode) to define Cutoffs and Symbols.



- After plate reading, open the **Symbols** data set to see the assigned symbols and the calculated cutoff values.

Selecting the Data to Interpret

The **Data to Interpret** setting represents the set of data to which the cutoffs will be applied. For example, if Data to Interpret is set to 'Concentrations', the cutoffs will be applied to the calculated concentrations.

KC4 presents for selection the applicable data sets that are made available by the current protocol definition.

Defining Cutoffs

KC4 supports the definition of up to 40 cutoffs. These are the criteria against which the plate data will be evaluated.

- Enter cutoff values or formulas in the **C.O.#. =** fields. Click or to see the next or previous 8 fields.
- Before defining cutoffs that reference **well identifiers,** ensure that all identifiers have been assigned under **Protocol|Plate Layout.**
- Cutoffs must be entered in **ascending** order. If entering formulas, the calculated results of the formulas must be in ascending order to be considered valid.

  For example, if Data to Interpret is set to 'Concentrations' and the cutoffs are defined as 'STD1', 'STD2', and 'STD3', then the calculated concentrations must be in ascending order, such as 10.000, 20.000, and 30.000.
- Each cutoff can contain any of the following:

  Numeric values (1.500, 85000, 2.0E-8)

  Well identifiers (NC, BLK, STD1)

  Well coordinates (A1 , H12, P24)

  A formula combining any of the above (CTL1/CTL2*100)

Cutoff Formulas

The following table contains valid symbols and functions for use within cutoff formulas:

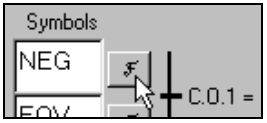
Symbol or Function	Description	Example
+	Addition	CTL1+0.100
-	Subtraction or negation	BLK-0.010
/	Division	CTL1/CTL2
*	Multiplication	STD1*0.10
( )	Represents inclusion	(CTL1/CTL2)*100
Well coordinates	The value of a specific well	A1/B1
MEAN[<ID>]	The mean of the specified well identifier	MEAN[POS1]
SD[<ID>]	The standard deviation of the specified well identifier	SD[BLK]
CV[<ID>]	The coefficient of variation of the specified well identifier	CV[STD1]
LOG(<x>)	Represents the LOG10 function	LOG(SPL10)
POW(<x>;<y>)	The value of <x> raised to the power of <y>	POW(STD1;3)
SQRT(<x>)	The square root of <x>	SQRT(A1*B1)
MIN(<x>;<y>;<z>;…)	The minimum of the defined variables	MIN(CV[CTL1]; CV[CTL2])
MAX(<x>;<y>;<z>;…)	The maximum of the defined variables	MAX(A1;B1;C1)

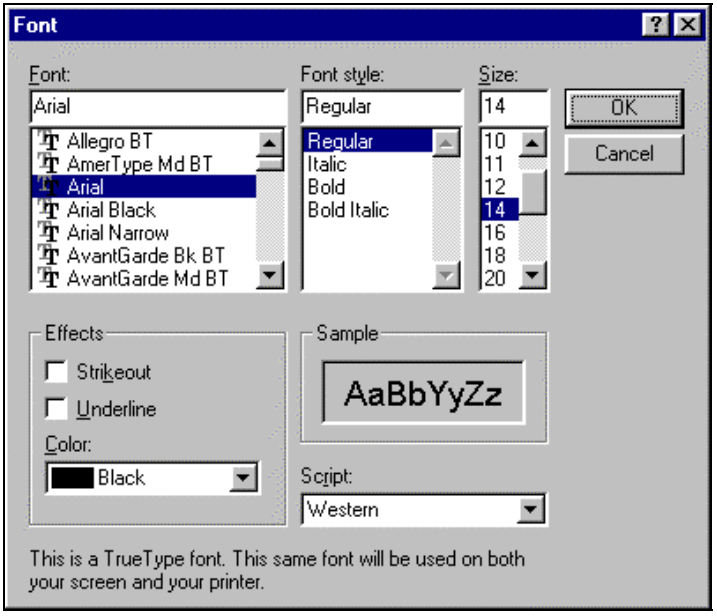
Defining Symbols

Symbols are assigned to wells after cutoff evaluation. After plate reading, open the 'Symbols' results data set to see the assigned symbols as well as the calculated cutoff values.

The number of available Symbols fields exceeds the number of defined cutoffs by one. During data reduction, if the value of a well is less than the first cutoff, the first symbol is assigned to the well. If the value of a well is greater than or equal to the first cutoff, but less than the second cutoff, the second symbol is assigned to the well, and so on. See *Results, Symbols* later in this manual for more information.

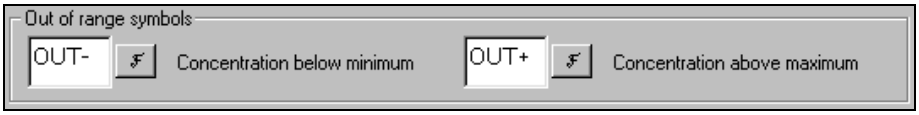
Each Symbol can contain up to 8 alphanumeric characters. Common symbols include NEG, EQV, POS, -, +. The appearance of each symbol can be customized by clicking its corresponding Font button:





- The default Font is MS Sans Serif Regular 10 point (or similar), in Black. KC4 presents for selection the fonts that are already installed on your PC (typically in your \Windows\Fonts folder.
- Change the Font, Font Style, Size, and color, as desired. Add Effects such as a Strikeout or an Underline. Symbols will appear on the results display and on printed reports as they are defined within this dialog.

Out-of-Range Symbols



When 'Data to Interpret' is set to 'Concentrations', the **Out of Range Symbols** fields are available at the bottom of the Cutoffs dialog.

Following data reduction:

- Wells with concentrations less than the minimum standard concentration are assigned the 'Concentration below minimum' symbol.
- Wells with concentrations greater than the maximum standard concentration are assigned the 'Concentration above maximum' symbol.
- **Note:** Use of the Extrapolation Factor may cause the upper and lower limits for concentration interpretation to be extended.

The default out of range symbols are OUT- and OUT+. Each Symbol field can contain 0 to 8 alphanumeric characters. The appearance of each symbol can be customized by clicking its corresponding Font button, as described in the previous section.

Cutoffs Evaluation

Cutoffs are evaluated during data reduction. If the cutoffs are in increasing order, symbol assignment is determined as follows:

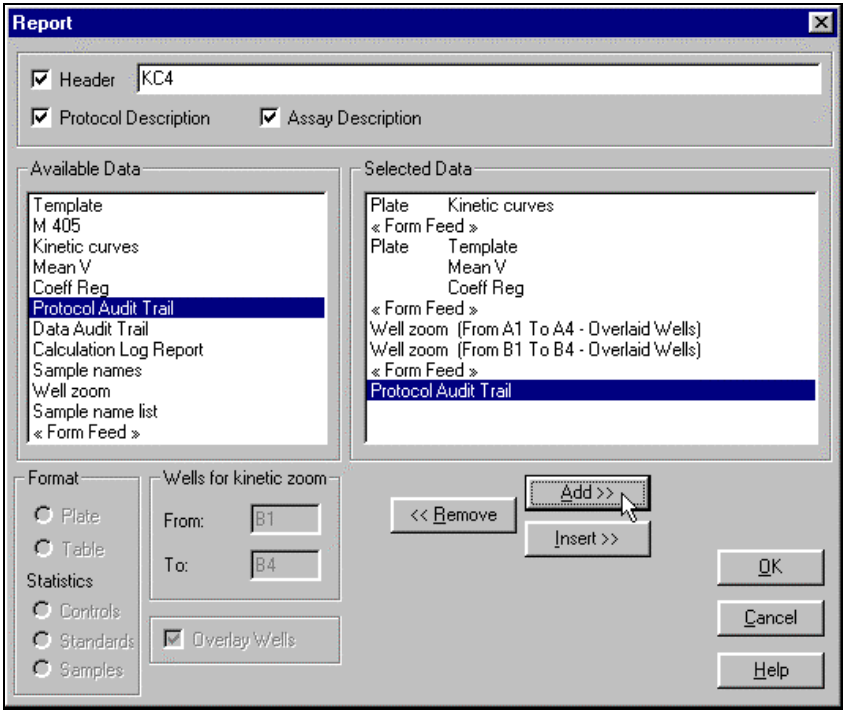
- Each well is evaluated against the first cutoff. If the value of the well is less than the criteria, then the first symbol is assigned to the well.
- If the value of the well is greater than or equal to the first cutoff, then it is evaluated against the next cutoff. If the value of the well is less than the next criteria, then the symbol immediately preceding the cutoff criteria is assigned to the well.
- This process continues until the last cutoff criteria is encountered. If the value of the well is less than the last criteria, then the symbol immediately preceding the last cutoff criteria is assigned to the well. If the value of the well is greater than or equal to the last criteria, then the symbol immediately following the last cutoff criteria is assigned to the well.

Reports

With KC4's standard reporting function you can customize the contents, and to some extent the appearance, of printed reports. All KC4 users have access to this standard function. Reporting through Microsoft® Excel 2000 and Word 2000 is also supported with KC4's PowerReports feature.

This section only discusses the standard reporting function (the **KC4** report engine). The report engine is selected under **Protocol|Options.**

Select **Protocol|Reports** to customize the report for the open protocol:



- Check **Header** and enter the text to appear at the top of every report.
- Check **Protocol Description** and **Assay Description** to include this information on reports.
- The **Available Data** window displays the data and information available for reporting. **Available Data** selectable items are dependent upon the type of protocol selected, as well as the defined Reading Parameters.
  - Click the left mouse button to select an individual item.
  - To select groups of like items, hold the **Ctrl** key down while highlighting them.
  - Click the **Add>>** button to append the item(s) to the **Selected Data** list.

- Click the **Insert>>** button to insert item(s) into the **Selected Data** list. Highlight the item in the Selected Data list *above which* the inserted item should appear.
- You can **drag and drop** selected items between the two lists.
- The **Form Feed** option inserts a page break.

Audit Trail Reports

If **Protocol Audit Trail** is selected, a list of all changes made to the protocol is included on the report.

Protocol Audit Trail

Date	User	Action	Comment
08/15/02 14:23:16	MAJ	File Created	
08/15/02 14:27:56	MAJ	Reading changed	Set reference wavelength to 280
08/15/02 14:32:33	MAJ	Plate Layout changed	Designed the plate layout
08/15/02 14:38:12	MAJ	Multi Plate Transformation changed	Adjusted Delta OD transformation
08/15/02 14:46:07	MAJ	Report changed	Designed the report template
08/15/02 15:11:23	MAJ	Signed for Authorship by Mary Ann Jones	
08/16/02 09:35:28	RMB	Signed for Review by Robert Michael Barnes	

If **Data Audit Trail** is selected, a list of all changes made to the data file is included on the report.

Data Audit Trail

Date	User	Action	Comment
08/19/02 16:21:18	MAJ	Protocol "DB:\Jones Mary A\Nucleic Acid\Phenol.prt" (08/17/02 10:21:15)	
08/19/02 16:23:32	MAJ	Plate read started	
08/19/02 16:24:27	MAJ	Sample Plate successfully read	
08/19/02 16:26:53	MAJ	File Created	
08/19/02 16:26:53	MAJ	Signed for Authorship by Mary Ann Jones	
08/19/02 16:48:22	MAJ	Options changed	Changed the weighting of standard deviation to 'N'
08/19/02 17:02:10	MAJ	Report changed	Designed the report template
08/20/02 09:25:38	RMB	Signed for Review by Robert Michael Barnes	

Calculation Log Report

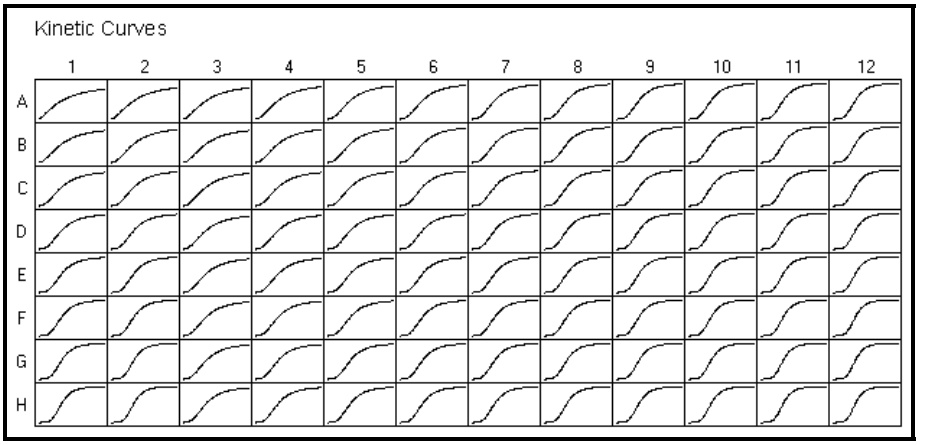
If **Calculation Log Report** is selected, warning(s) appear on reports if a special event occurs during the calculations. Events can include a failure during the calculation of a transformation, the standard curve, or cut-offs:

Calculation Log Report

Calculation Warnings
Data In not available for Multi-Plate Transformation #1
The 4 parameter fit is not convergent. It is probably not the most adequate fit.
Some cutoffs could not be calculated. Symbols could not be determined.

Kinetic Curves Report

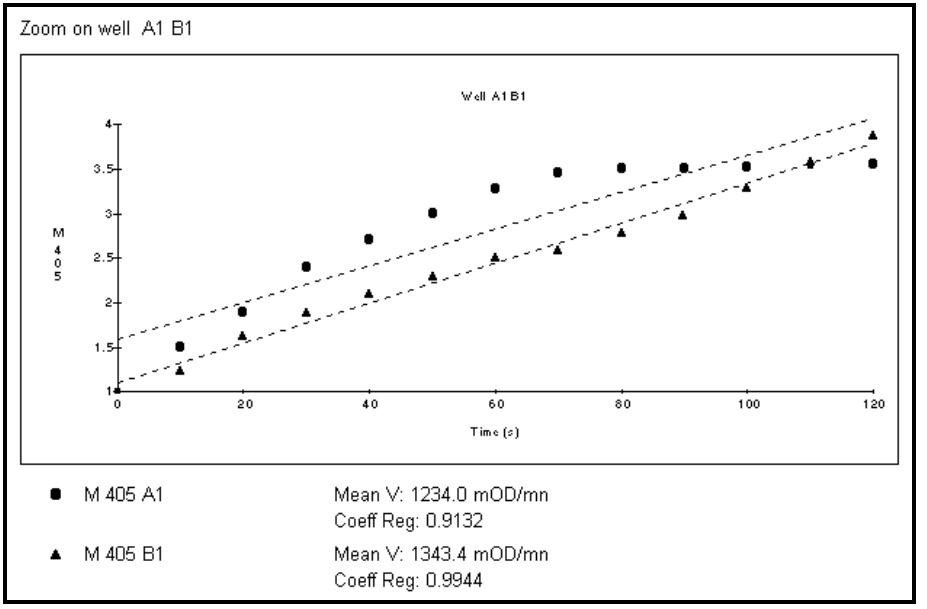
The **Kinetic Curves** report shows the kinetic curve in every well, in a plate format. For example:



Well Zoom Report

Each **Well Zoom** report contains the kinetic curve for one well.

- To specify a Well Zoom report, highlight **Well Zoom** in the Available Data box, enter the desired **Wells for kinetic zoom,** then click the Add button.
- Up to **8** kinetic wells can be overlaid in a single graph. Select the **Wells for kinetic zoom,** then click **Overlay Wells.**



Validation Report

The **Validation** report contains every validation condition defined for the protocol and its resulting status.

- The possible status values are **Verified, Not Verified,** and **Could Not Be Evaluated.**

VALIDATION

Data type : M 405

	Condition	Status
1	0.000 < BLK < 0.200	VERIFIED

Data type : M 405 Corr.

	Condition	Status
1	0.000 < NEG1 < 0.500	VERIFIED
2	2.500 < POS1 < 3.000	**NOT VERIFIED**

Cutoffs Report

The **Cutoffs** report lists the defined cutoffs and their calculated results.

CUTOFFS – Concentrations

#	Value	Formulae
1	0.6116	STD2*0.8
2	17.876	STD4
3	61.007	STD6*1.2

- In the example above, cutoffs were based on the **Concentrations.**

Sample Name List Report

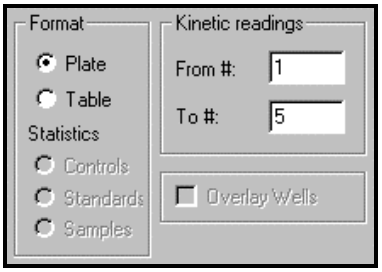
The **Sample Name List** contains all sample names defined for the plate.

Sample Name List

#	Name
1	SMP0001
2	SMP0002
3	SMP0003
4	SMP0004
5	SMP0005
6	SMP0006
7	SMP0007
8	SMP0008
9	SMP0009
10	SMP0010

Plate vs. Table Format

Depending on the nature of the item chosen under **Available Data,** information can be reported either in **Plate** format or in **Table** format.



- With the **Plate** format selected, data will print in a matrix format corresponding with the plate layout.
- Data for **individual kinetic reads** can be printed in the Plate format. Select the type of data (e.g., Meas. OD, Delta OD), then set the range of reads to include in the report using the **From #** and **To #** fields.
- The **Table** format is available for kinetic and scanned data. All kinetic or scanned readings for one or multiple wells. Set the range of wells to include in the report using the **From** and **To** fields.

Statistics Report

Statistical expansion of the data can be reported. This information is specifically related to the **Identifiers,** e.g. controls, standards or samples.

- To specify a **Statistics** report, select an item from the **Available Data** box, click the **Controls, Standards,** or **Samples** radio button in the Statistics box, then click the **Add** button.

STATISTICS – M 405

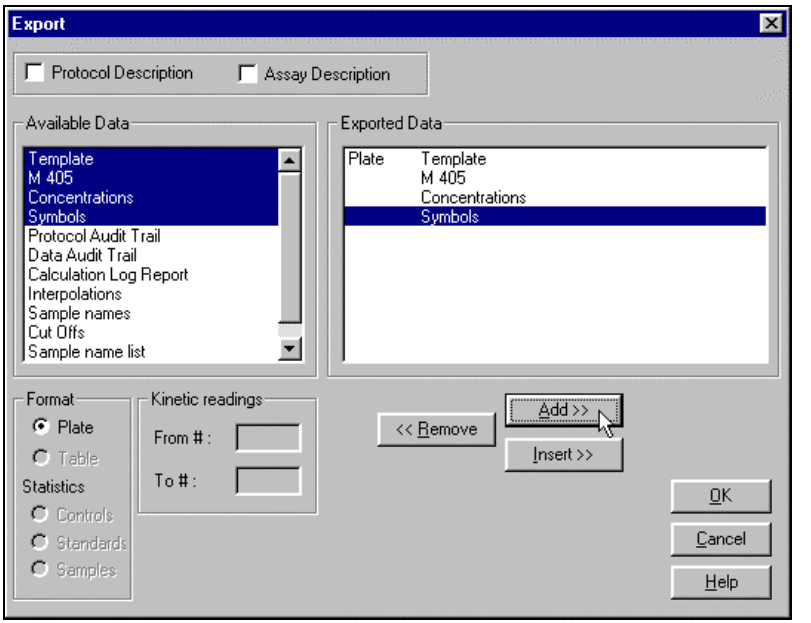
Identif.	Name	Conc\Dil	Well	405	Nb	Mean	Std Dev	CV(%)
SPL1	SMP0001	1.000	A1	0.234	3	0.2370	3.606E-3	1.5213
			A2	0.236				
			A3	0.241				
SPL2	SMP0002	1.000	B1	1.495	3	1.4987	3.215E-3	0.2145
			B2	1.500				
			B3	1.501				
SPL3	SMP0003	1.000	C1	0.887	3	0.8847	2.517E-3	0.2845
			C2	0.882				
			C3	0.885				
SPL4	SMP0004	1.000	D1	0.028	3	0.1117	0.1423	127.45
			D2	0.031				
			D3	0.276				

Export

With KC4's standard exporting function you can customize the contents of export files. All KC4 users have access to this standard function. Exporting through Microsoft® Excel 2000 and Word 2000 is also supported with KC4's PowerReports function.

This section only discusses the standard exporting function (the **KC4** report engine). The export engine is selected under **Protocol|Options.**

Select **Protocol|Export** to customize the export file for the open protocol:



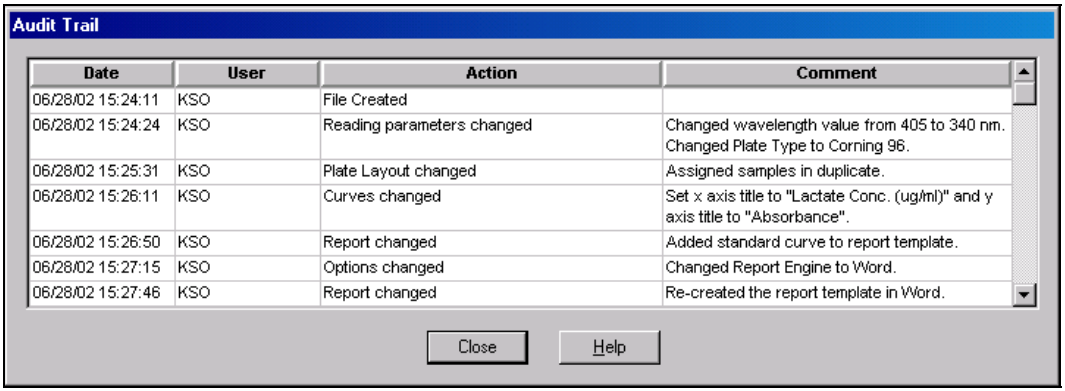
- Check **Protocol Description** and **Assay Description** to include this information in the file.
- The **Available Data** window displays the data and information available for exporting. **Available Data** selectable items are dependent upon the type of protocol selected, as well as the defined Reading Parameters.
  - Select an *individual* item by clicking it with the left mouse button. To select *groups* of like items, hold the **Ctrl** key down while highlighting them.
  - Click the **Add>>** button to transfer the item(s) to the bottom of the **Selected Data** list.
  - Click the **Insert>>** button to insert item(s) into the **Selected Data** list. Highlight the item in the **Report** field *above* which the inserted item should appear.
- See "Formats" in *System Menu, Setup* for information on choosing the text delimiter.

Protocol Audit Trail

This feature only applies to KC4 Signature

Every protocol file has a **Protocol Audit Trail** associated with it. This audit trail automatically records **events** performed on the file since the file was created (first saved). Each event records an **action** such as "Plate Layout changed" or "Reading parameters changed" and includes a date and time stamp, the ID of the user logged in at the time, and any comments that were entered by the user when the action was performed. The audit trail cannot be edited and it remains a permanent part of the file for the life of that file (that is, if the file is deleted, the audit trail is also deleted).

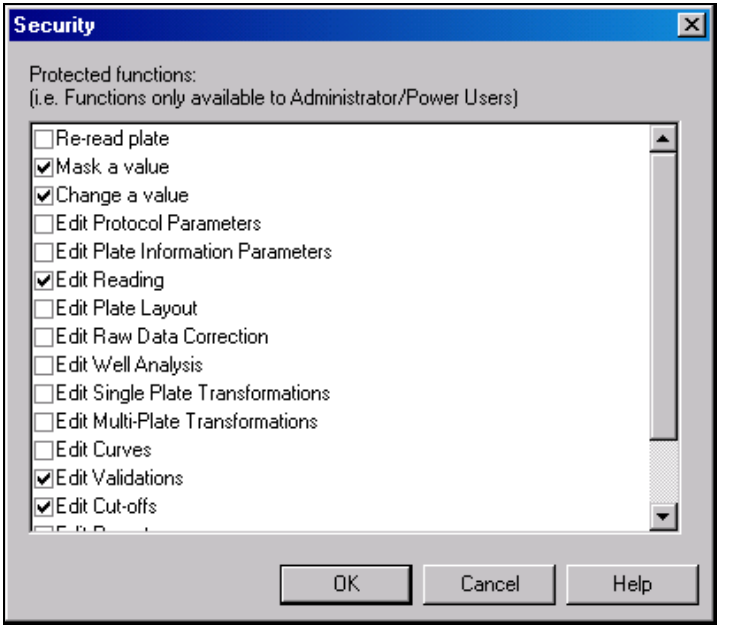
To view a protocol's audit trail, select **Protocol|Audit Trail:**



- Events are sorted by date/time stamp and include the ID of the user logged in at the time, the action taken, and any comments that were entered by the user when the action was performed.
- The contents of the protocol audit trail can be copied to the Clipboard by highlighting the desired cells and pressing Ctrl+C.
- The contents of the protocol audit trail **cannot** be edited or deleted.

Security

KC4 provides the ability for the Administrator to protect the use of certain protocol/data functions by unauthorized users. To view or modify the protected functions associated with a particular protocol, select Protocol|Security (advanced function).



Checked ☑ functions may not be performed by **unauthorized users.**

- With KC4 v3.4, an unauthorized user is any user other than the System Administrator.
- With KC4 *Signature*, an unauthorized user is one who is designated as a Standard User.

The following list includes all protocol-level protectable functions and describes the behavior the unauthorized user can expect from KC4 for each function when it is protected:

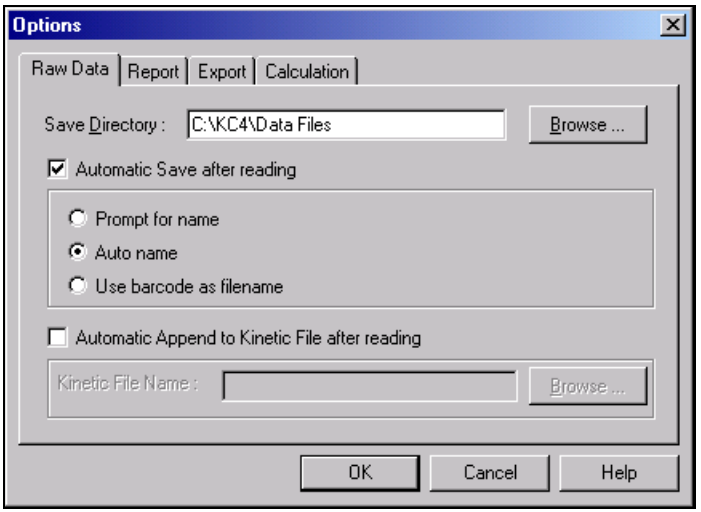
- **Re-read plate.** The user cannot re-read a plate if one is already open, because re-reading a plate deletes the initial values. The user must first select Data|New Plate and then Data|Read Plate.
- **Mask a value.** Within the Results window, the user cannot click the Mask box to exclude wells from calculations.
- **Change a value.** Within the Results window, the user cannot click the Change box to change any measurement values.
- **Edit Protocol Parameters.** If this box is checked, the user cannot edit *any* protocol parameters.
- **Edit <individual protocol parameter>.** The user cannot modify the particular protocol parameter. For example, if 'Edit Plate Layout' is protected, the user cannot define or assign new well IDs, expected concentration values, or dilution values. The user can, however, select Protocol|Plate Layout to view the layout.

Protocol Options

Select **Protocol|Options** to customize various options for this protocol, including automatic file saving, report printing, and data exporting. Reporting, exporting, and calculation options can also be modified here if the desired options are different than the default ones specified under **System|Setup.**

Raw Data

Select **Protocol|Options|Raw Data** to specify automatic save and append options for raw data.

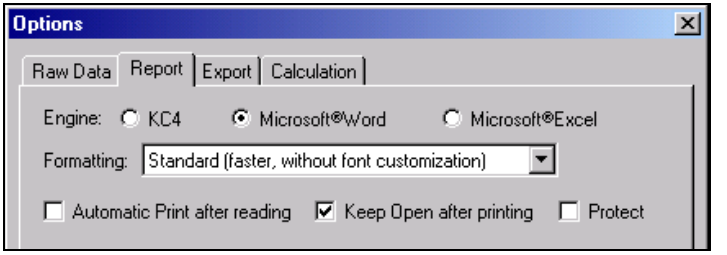


- Enter the **Save Directory** in which to store plate data files (*.pla and *.glb) associated with this protocol. Click **Browse** to search for and select the desired location.
  - **Note:** If files will not be saved automatically (see below), this location can be changed manually by selecting **Plate|Save** or **Plate|Save As.**
- Check **Automatic Save after reading** to automatically initiate the save plate data file function when the read is complete.
  - Select **Prompt for Name** to prompt the operator to manually enter the file name after the plate read, or select **Auto Name** to let KC4 automatically name the file, using the system date and a sequence number. The use of 'Auto Name' ensures a unique file name each time.
- If the reader is equipped with a barcode scanner and "Read Barcode" is selected under Protocol|Reading, the **Use barcode as filename** option is available. Select this option to automatically save the data file using the barcode name.

- Select **Automatic Append to Kinetic File after reading** to append the current data to an existing data file. This feature allows you to perform several endpoint reads on one plate. You can temporarily remove the plate to incubate it externally, or perhaps to add reagents.
  - Enter the target directory and file name in the **Kinetic File Name** field. Click the **Browse** button to search for and select the desired file.
  - **Note:** 'Automatic Append to Kinetic File' is not available if the protocol specifies pathlength correction or the pre-reading of a blank plate.

Report

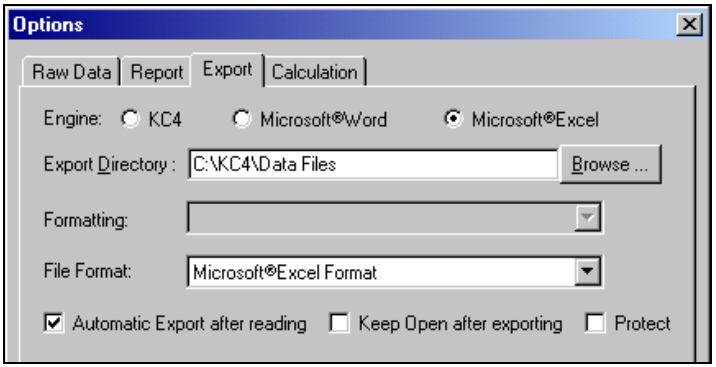
Select **Protocol|Options|Report** to select the reporting engine and to set printing options.



- Select the **Engine** for designing report templates:
  - **KC4:** This is the standard method for designing reports that KC4 has always provided. The contents are customizable but the appearance is completely controlled by KC4 (you cannot change the font style or size, for example).
  - **Word:** If your computer has Microsoft® Word 2000 installed on it, you can design the report using Word and take advantage of all its formatting features.
  - **Excel:** If your computer has Microsoft® Excel 2000 installed on it, you can design the report using Excel and take advantage of all of its formatting features. In addition, you can create custom Excel formulas that reference KC4 data.
- If you chose Word as the engine, choose a **Document Formatting** option to tell KC4 how to prepare documents for printing and print previewing.
  - If **Standard** is selected, document formatting is faster because KC4 does not apply any custom font characteristics from the Plate Layout or Cutoffs dialogs. Well IDs and cutoff symbols are printed using just the default font characteristics.
  - If **Enhanced** is selected, document formatting is slower because KC4 *does* apply custom font characteristics to Well IDs and cutoff symbols.
- Select **Automatic Print after reading** to automatically print reports when the plate read is complete. This is available for all reporting engines.
- Select **Keep Open After Printing** to keep Word or Excel open after the print preview or after printing, to allow for *temporary* formatting adjustments. Note: To make permanent changes, modify the report via Protocol|Report.
  - If Keep Open After Printing is enabled, the **Protect** option can be enabled to prevent unauthorized changes to the document.

Export

Select **Protocol|Options|Export** to select the export engine and define related parameters.

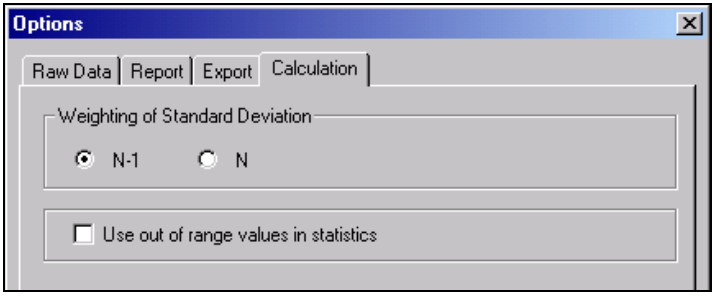


- Select the **Engine** for designing export templates:
  - **KC4:** This is the standard method for designing export files that KC4 has always provided. The contents are customizable but the appearance is completely controlled by KC4 (you cannot change the font style or size, for example).
  - **Word:** If your computer has Microsoft® Word 2000 installed on it, you can design export file templates using Word and take advantage of all of its formatting features.
  - **Excel:** If your computer has Microsoft® Excel 2000 installed on it, you can design export file templates using Excel and take advantage of all of its formatting features. In addition, you can create custom Excel formulas that reference KC4 data.
- If you chose Word as the engine, choose a **Document Formatting** option of Standard or Enhanced.
  - If **Standard** is selected, document formatting is faster because KC4 does not apply any custom font characteristics from the Plate Layout or Cutoffs dialogs. Well IDs and cutoff symbols are printed using just the default font characteristics.
  - If **Enhanced** is selected, document formatting is slower because KC4 *does* apply custom font characteristics to Well IDs and cutoff symbols.
- Enter the **Export Directory** in which to store exported files associated with this protocol. Click **Browse** to search for and select the desired location. Note: Select the export text file **delimiter** under **System|Setup.**
- Select **Automatic Export after reading** to automatically export plate data to a file when the read is complete.
  - If 'Automatic Save after reading' is selected, the export file name will match the manually entered file name, the automatically generated file name, or the barcode, depending on the automatic save parameters, and will have a .txt extension. If 'Automatic Save after reading' is not selected, the export file name will be NONAME.TXT.
- If the export engine is Excel, choose a **File Format** of Excel, Excel CSV, or Excel Text.

- Select **Keep Open After Exporting** to keep the Word or Excel application open after the preview or after exporting, to allow for *temporary* formula and/or formatting adjustments. (To make permanent changes, modify the export file via Protocol|Export.)
  - If Keep Open After Exporting is enabled, the **Protect** option can be enabled to prevent unauthorized changes to the document.

Calculation

Select **Protocol|Options|Calculation** to select a weighting of standard deviation and tell KC4 whether or not to include out-of-range values in statistics calculations.



- Select an option for the **Weighting of Standard Deviation:**

  N-1 Used in calculations where the data is assumed to represent a sample from a larger population.

  N Used in calculations where the data is assumed to represent the total population from which the sample was taken.

- Choose whether or not to **use out of range values in statistics.** Out of range values are those values reported as '<xxxx' or as '>xxxx'.

  Example: SPL1 has three replicates reported with the following concentrations:

  well A1 : 10.230
  well A2 : <10 (out of range value)
  well A3 : 10.450

  If the option is *not* selected, the software will not use the concentration of well A2 in the statistic calculations. Therefore the mean, standard deviation and CV of SPL1 will be 10.34, 0.155, and 1.5%, respectively. If the option *is* selected, concentration of well A2 is taken into account (the value of 10 is used in the statistic calculations). Therefore the mean, standard deviation and CV of SPL1 will be reported as <10.22, 0.225, and 2.2%, respectively.

  Important! Exercise caution when enabling this option, as it can lead to biased results particularly for standard deviation and CV. As a warning, statistic results arising out of the use of this option are displayed in italic in the report.

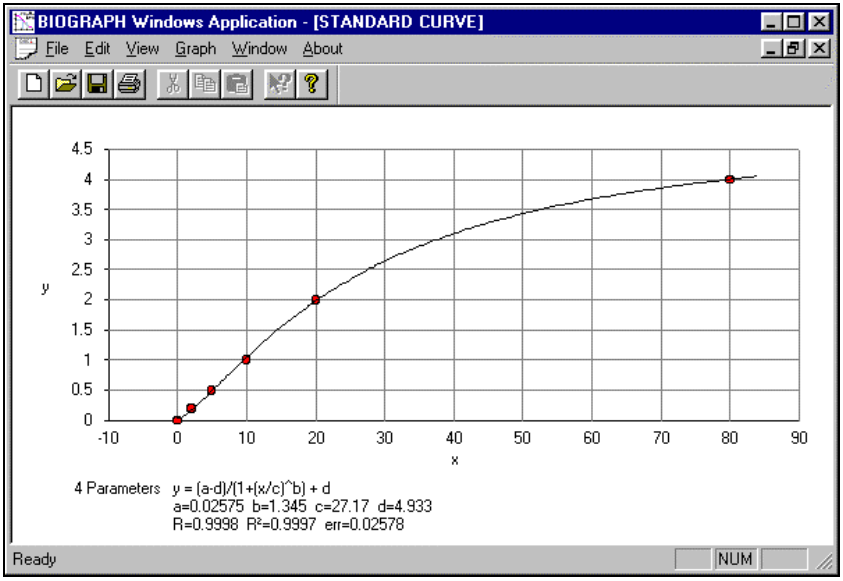
Display Menu

Overview

The **Display** menu allows you to check the verification status of your data and/or to activate the Biograph module to view, adjust, and otherwise interact with test results as displayed on a curve or set of curves.

Curve(s)

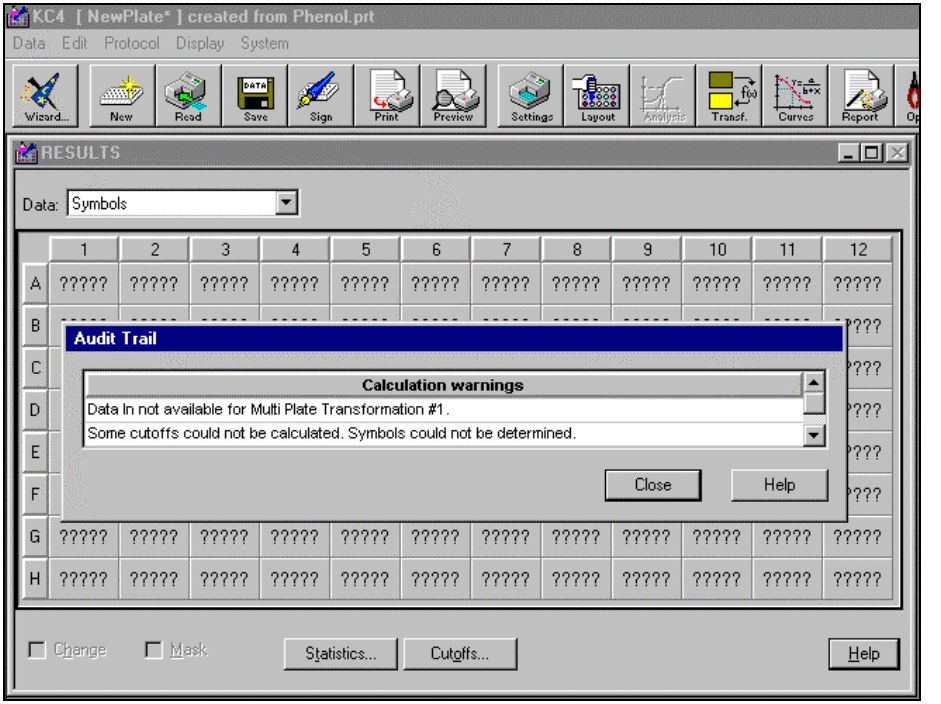
Select **Display|Curves** to load Biograph and view the curve or curves generated using the current plate data. This option is available only if the protocol defines a standard curve or dilution curves.



- When **Biograph** is loaded, every curve calculated for the current plate appears in a window. See "Biograph" for information on changing the curve fitting method, deleting standard points, printing the graph, and more.
- To quit Biograph and return to KC4, select **File|Exit.**

Calculation Warnings

Select **Display|Calculation Warnings** to view a list of data reduction calculation warnings issued by KC4 against the current set of data. (If this list is empty, KC4 found no errors with the calculations.)


KC4 [NewPlate*] created from Phenol.prt
Data Edit Protocol Display System
Wizard... New Read Save Sign Print Preview Settings Layout Analysis Transf. Curves Report
RESULTS
Data: Symbols
1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H
Audit Trail
Calculation warnings
Data In not available for Multi Plate Transformation #1.
Some cutoffs could not be calculated. Symbols could not be determined.
Close
Help
Change
Mask
Statistics...
Cutoffs...
Help

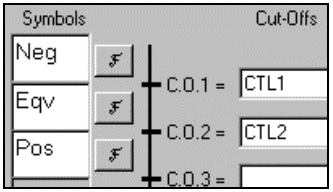
Warnings and their probable causes include:

- **At least one validation condition failed.**

  The protocol defines validation conditions and one or more conditions has failed. Select Display|Validation Results to see the current status of each condition.

- **Cutoffs are not in increasing order. Symbols can not be determined.**

  The protocol defines cutoff criteria, the results of which are not in increasing order and therefore Symbols (+, -, Pos, Neg, etc.) cannot be determined. For example, if criteria is defined like this:



  and if CTL1=2.050 and CTL2=1.432, cutoffs are not in increasing order. Select Protocol|Cutoffs to view/modify the criteria.

- **Data In not available for Multi Plate Transformation #<1, 2, or 3>**

  A multi plate transformation references a data set that no longer exists. Select Protocol|Multi Plate Transformation to choose another data set and then recalculate results.

- **Data In not available for Single Plate Transformation #<1, 2, or 3>**

  A single plate transformation references a data set that no longer exists. Select Protocol|Single Plate Transformation to choose another data set and then recalculate results.

- **Data to interpolate are not available. Standards could not be calculated.**

  The data set specified for curve fit calculation may no longer exist. Select Protocol|Curves, Curve Description. If the Data to Interpolate field contains asterisks, this is the case. Choose another data set and recalculate results.

- **Data to process <#> not available for kinetic calculations.**

  The data set specified for kinetic analysis may no longer exist. Select Protocol|Kinetic Analysis. If the Data to Process (1 or 2) field is empty, this is the case. Choose another data set and recalculate results.

- **Log Axis cannot be used with a negative or null value.**

  KC4 was unable to calculate the curve because one or both Axes is set to calculate logarithmically and an input value (such as a standard OD) is negative or null. Select Protocol|Curves, Curve Fit to view the current curve parameters.

- **Not enough valid standards.**

  Each curve fit requires a minimum number of valid standards; either not enough standards are defined in the plate layout, or following results calculation too many standards were determined to be invalid.

- **Some cutoffs could not be calculated. Symbols could not be determined.**

  The cutoff criteria may reference a data set or well ID that no longer exists, or a referenced well ID may contain an invalid value. Select Protocol|Cutoffs to examine the current criteria.

- **The archived curve could not be loaded. Path is probably wrong.**

  This message was likely preceded by a "<pathname><filename>.gph was not found" message. If this is the case, the protocol references a stored curve in the location specified in that message. Select Protocol|Curves, Curve Description. Under Curve Origin, if File is selected click Browse to locate the appropriate .gph stored curve file.

- **The curve fit method is not compatible with the standards. The curve could not be calculated.**

  Every curve fit requires a minimum number of valid standards; there may not be enough valid standards to support the current curve fit. Select Protocol|Plate Layout to add more standard wells or select Protocol|Curves to choose another curve fit and then recalculate results.

- **The curve is not strictly increasing nor strictly decreasing.**

  The curve is ambiguous; more than one X value was calculated for a particular Y value (ex. more than one concentration was calculated for at least one OD/FU/LU).

- **There must be at least two valid standards.**

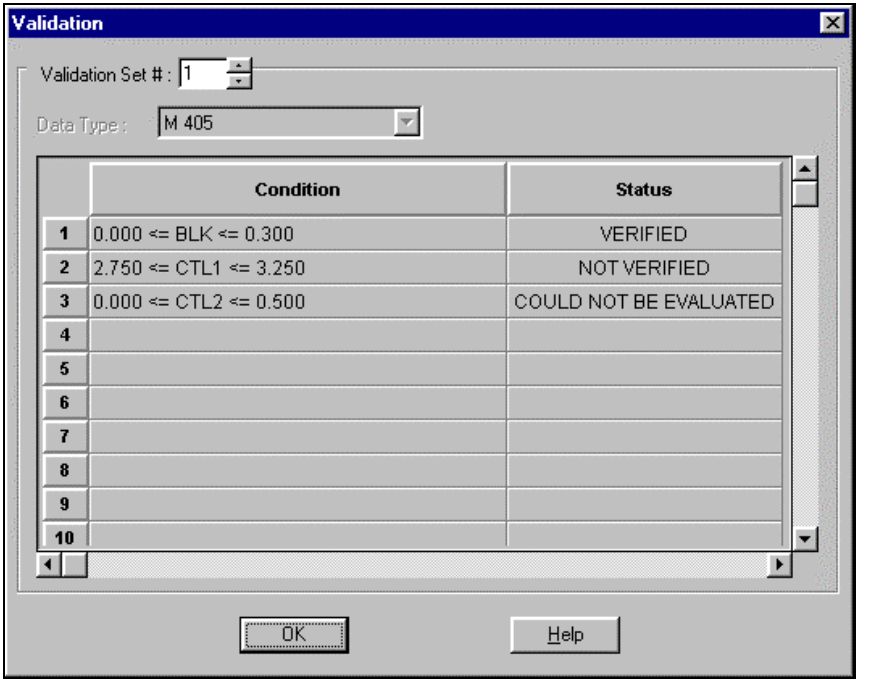
  The plate currently contains only one valid standard and at least two are required to calculate the curve. Check to see if any of the standard wells is currently masked or contains an out-of-range or another non-numeric notation.

- **Values out of range. Log Axis cannot be calculated.**

  KC4 was unable to calculate the curve because one or both Axes is set to calculate logarithmically and an input value (such as a standard OD) is out of range. Select Protocol|Curves, Curve Fit to view the current curve parameters.

Validation Results

Selecting **Display|Validation Results** shows the current status of each of the **Validation** conditions defined in the protocol:



- The **Condition** column lists the Validation criteria defined in the protocol.
- The **Status** column tells you whether or not each Condition has been met:

  VERIFIED: The condition has been met.

  NOT VERIFIED: The condition has not been met and results are therefore suspect.

  COULD NOT BE EVALUATED: The condition could not be evaluated. This may occur if, for example, a condition refers to an identifier with masked replicate values.

- **Important!** If a validation condition receives the status NOT VERIFIED then the results are suspect. The responsibility for any masking or editing of the data lies with the user.

System Menu

Overview

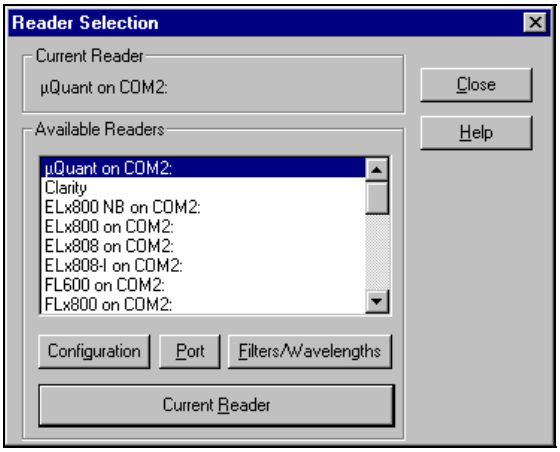
The options available under the **System** menu allow you to:

- Select the current reader and then set and test communication parameters.
- Run an optics test or a universal plate test on the attached microplate reader and view/print/store results.
- Configure security settings, set up user accounts, and define protected functions.
- Log into and out of KC4.
- Set protocol default settings, including report and export engine, and the weighting standard deviation.
- Set global parameters, including the import/export text separator and the time format for kinetics.
- Customize the arrangement of the toolbar buttons.
- Create and maintain microplate measurement information.

Reader Selection

Select **System|Readers** to select or change the current reader.

Important! Be sure to set and test communication parameters *before* attempting to read a microplate.



- Choose a reader from the **Available Readers** list, then define the Configuration, Port, and Filters/Wavelengths parameters.
- Click **Port** to define communication parameters including Port number, Transmission Speed, Data Bits, Stop Bits, and Parity.
- Click **Filters/Wavelengths** to align KC4's wavelengths table with the reader's internal table.
- If applicable, click **Configuration** to define the optical probe size so that it matches the current hardware configuration.
- When all parameters are set, click the **Current Reader** button. KC4 will attempt to communicate with the attached reader. If communication fails, KC4 provides information for solving the problem. Follow the instructions and then try again.

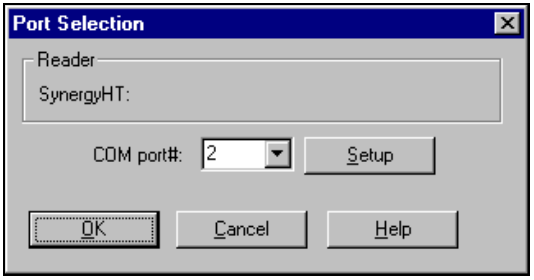
Note: If the current reader is set to "Clarity", the **Configuration** and **Port** buttons are dimmed and inaccessible because configuration parameters and port settings can only be accessed through the Clarity™ PC software. The **Filters/Wavelengths** button is dimmed and inaccessible because it does not apply to the luminometer.

Note: If the computer has more than one COM port, you can control more than one instrument at a time by running **multiple instances** of KC4. See "Controlling Multiple Instruments Simultaneously" at the end of this section for more information.

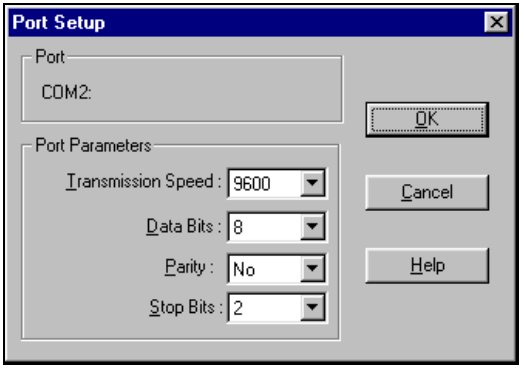
Port Selection

Use the **Port Selection** dialog to select the COM port number (1 to 9), and to access additional communication parameters.

Clarity™ Microplate Luminometer users: Port settings can only be accessed through the Clarity PC software. See the Clarity Operator's Manual for more information.



- Click the drop-down arrow and select the number that represents the **COM Port** KC4 should use when communicating with the reader.
- Click **Setup** to define additional communication parameters.



  - **Important!** It is the user's responsibility to define the correct parameters, otherwise communication errors will likely occur. Ensure the communication settings match those defined on the reader.
  - **Transmission speed:** Values commonly accepted by readers are between 2400 and 9600 bps.
  - **Data Bits:** Options range from 4-8 bits.
  - **Parity:** Options include No, Odd, Even and Mark.
  - **Stop bits:** Options include 1, 1.5 and 2 bits.

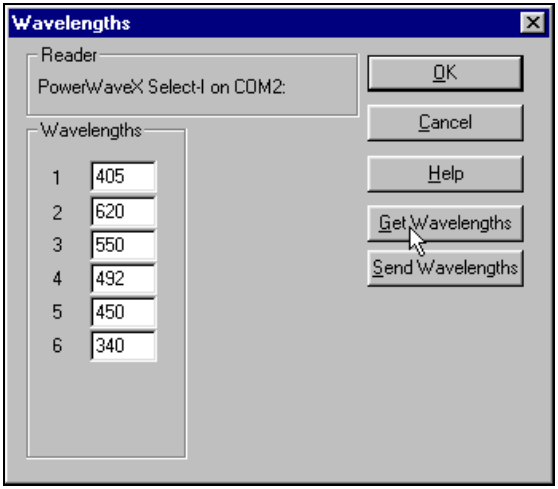
Filters/Wavelengths

Use the **Wavelengths** dialog to ensure that KC4's wavelengths table is aligned with the reader's internal table.

Note: It is essential that you conform to the specific Bio-Tek reader procedures for altering the configuration. When using filter-based readers, it is your responsibility to ensure that correct filters are allocated to their respective positions otherwise incorrect data will result.

Clarity™ Microplate Luminometer users: KC4's Filters/Wavelengths options do not apply to the luminometer.

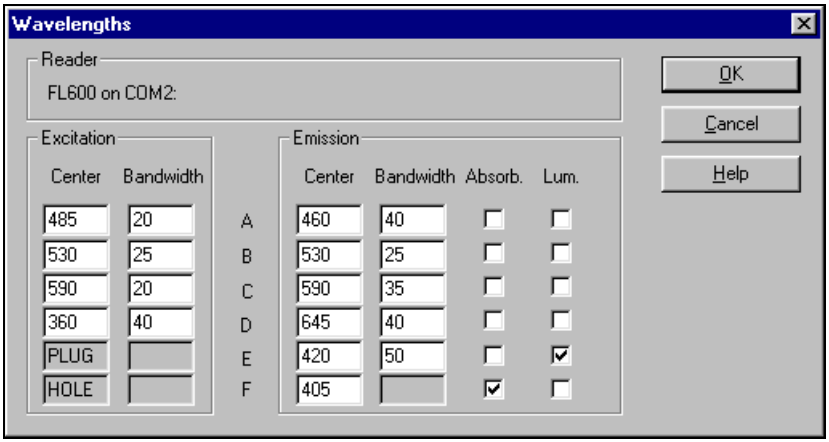
ELx-Series / PowerWave Series / µQuant



Note: To exchange wavelength information between KC4 and the instrument, the two must be communicating. The currently selected reader and COM port are displayed at the top of this dialog. If you need to change the COM port, click Cancel now to exit this dialog and then click the Port button.

KC4's currently-defined **Wavelength** values are displayed in nanometers in fields 1 through 6. The settings here will be made available as default selections within the Protocol|Reading Parameters dialog.

- To change, download, and calibrate wavelength values, entered the desired values in these fields and then click **Send Wavelengths.** The values will be downloaded to the instrument, overwriting its existing wavelength table.
- To retrieve wavelength values from the instrument, click **Get Wavelengths.** The values will be uploaded to KC4, overwriting the values in this dialog.



KC4 cannot automatically retrieve the wavelength information from the FL600, therefore this information must be entered manually. Consult the **Order Entry Form** that was shipped with the unit to determine which filters were originally installed. If this information cannot be found, note the reader serial number then contact Bio-Tek Instruments.

Tip: If the exact filter setup is unknown, you can remove the FL600's shroud to examine the filters in the Excitation and Emission filter wheels. The filters should be imprinted with the wavelength and bandwidth. Refer to the FL600 Hardware Guide for instructions.

- **A - F** represent the 6 filter positions on the Excitation and Emission filter wheels. **Excitation** refers to the light directed at the sample in the well. **Emission** refers to the light emitted by the sample in the well.
- **Center** is the center wavelength, in nanometers, of the installed filter.
- **Bandwidth** is the filter bandpass width, in nanometers (nm), of the installed filter (does not apply to absorbance filters).

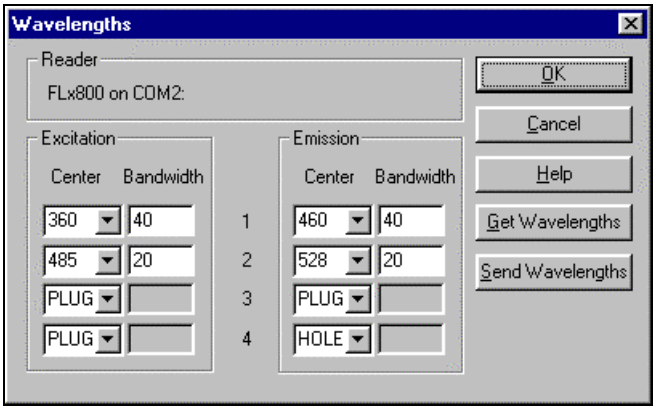
  Example: The Excitation/Center setting of 485/20 refers to a center wavelength of 485 nm with a the total bandpass of 20 nm. This combination will transmit light from 475 to 495 nm (10 nm on either side of the center).
- A check in the **Absorb.** column informs the software that the corresponding filter is an absorbance filter. If at least one Absorb. box is checked, the 'HOLE' notation appears in the Center field for Excitation position F.

  Note: It is not advised to check Absorb. for fluorescence filters because the bandpass is not fixed at 8 to 10 nm but can be as wide as 50 nm or more. Some fluorescence filters with narrower bandpasses, however, might be able to function as absorbance filters.
- A check in the **Lum.** column informs the software that the corresponding filter will be used for luminescence reading. If at least one Lum. box is checked, the 'PLUG' notation appears in the Center field for the Excitation position E.

  Note: When a Lum. box is checked, its corresponding Bandwidth field is not disabled, although the bandwidth is not used during luminescence plate reading.

FLx800™

The FLx800 has one four-position excitation wheel and one four-position emission filter wheel. Each filter wheel can contain any combination of filters, plugs, and empty locations, depending on assay requirements. Use this dialog to specify the exact contents of each filter wheel. The settings here will be made available as default selections within the Protocol|Reading Parameters dialog.



Important! It is essential that you conform to the specific Bio-Tek instrument procedures for altering the filter wheel configuration. The FLx800 does not automatically detect which filters (if any) are installed. Therefore, it is your responsibility to ensure that KC4's wavelength table exactly matches the contents of each filter wheel. For instructions for physically changing filters, see the FLx800 operator's manual.

Note: To exchange wavelength information between KC4 and the reader, the two must be communicating. The currently selected reader and COM port are displayed at the top of this dialog. If you need to change the COM port, click Cancel to exit this dialog now and then click the Port button.

To change the current settings and download them to the instrument:

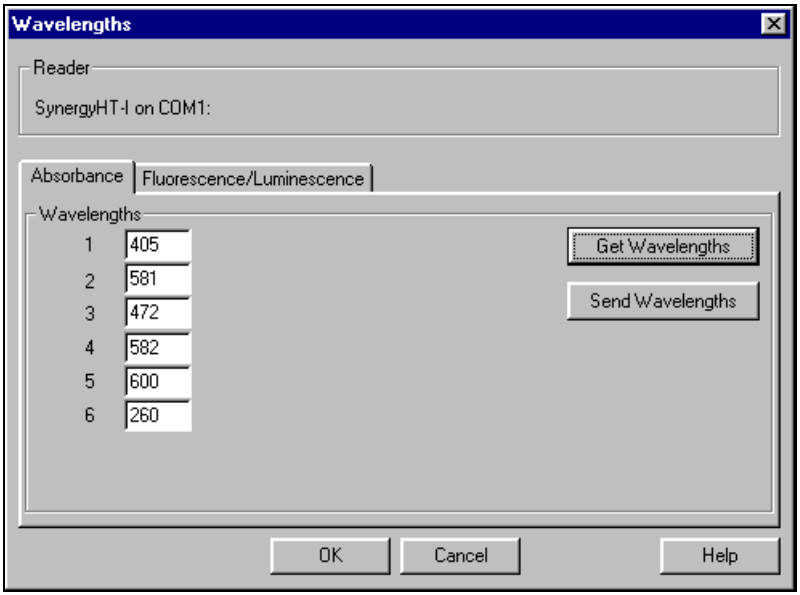
- Enter wavelength values in the **Center** fields, or use the drop-down boxes to select **PLUG** or **HOLE.** 'PLUG' indicates a solid plug in the filter wheel. A plug in the Excitation filter wheel is typical for luminescence assays, to prevent ambient light from entering the measurement chamber. 'HOLE' indicates an empty location in the filter wheel. An empty location in the Emission filter wheel is typical for luminescence assays.
- For each Center wavelength value, enter its accompanying **Bandwidth.** Note: This information is printed on the side of each filter. See the FLx800 operator's manual for details. 'Bandwidth' is the filter bandpass width, in nanometers, of the installed filter. For example, the Center/Bandwidth combination of 485/20 will transmit light from 475 to 495 nm (10 nm on either side of the center).
- When all values have been entered, click **Send Filters.** The values will be downloaded to the instrument, overwriting its existing wavelength table.

To retrieve filter wheel settings from the instrument:

- Click **Get Filters.** The values will be uploaded to KC4, overwriting the values in this dialog.

Synergy™ HT (Absorbance)

The Synergy HT performs absorbance reads in the range of 200 to 999 nm. Use this dialog to specify and calibrate six wavelengths to be made available as default selections within the Reading Parameters dialog.



Note: To exchange wavelength information between KC4 and the instrument, the two must be communicating. The currently selected reader and COM port are displayed at the top of this dialog. If you need to change the COM port, click Cancel to exit this dialog now and then click the Port button.

KC4's currently defined wavelength values are displayed in fields 1 through 6.

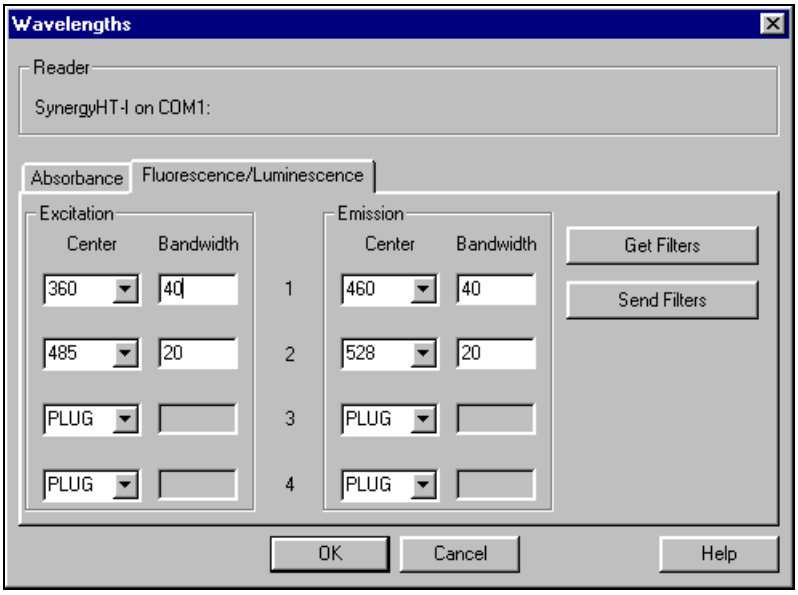
- To change, download, and calibrate wavelength values, entered the desired values in these fields and then click **Send Wavelengths.** The values will be downloaded to the instrument, overwriting its existing wavelength table.
- To retrieve wavelength values from the instrument, click **Get Wavelengths.** The values will be uploaded to KC4, overwriting the values in this dialog.

Synergy™ HT (Fluorescence/Luminescence)

The Synergy HT instrument has one four-position excitation wheel and one four-position emission filter wheel. Each filter wheel can contain any combination of filters, plugs, and empty locations, depending on assay requirements. Use this dialog to specify the contents of each filter wheel. The settings here will be made available as default selections within the Reading Parameters dialog.

Important! It is essential that you conform to the specific Bio-Tek instrument procedures for altering the filter wheel configuration. The Synergy HT does not automatically detect which filters (if any) are installed. Therefore, it is your responsibility to ensure that KC4's wavelength table exactly matches the contents of each filter wheel. For specific instructions on changing filters, see the Synergy HT Operator's Manual.

Note: To exchange wavelength information between KC4 and the instrument, the two must be communicating. The currently selected reader and COM port are displayed at the top of this dialog. If you need to change the COM port, click Cancel to exit this dialog now and then click the Port button.



To change the current settings and download them to the instrument:

- Enter wavelength values in the **Center** fields, or use the drop-down boxes to select **PLUG** or **HOLE.** 'PLUG' indicates a solid plug in the filter wheel. A plug in the Excitation filter wheel is typical for luminescence assays, to prevent ambient light from entering the measurement chamber. 'HOLE' indicates an empty location in the filter wheel. An empty location in the Emission filter wheel is typical for luminescence assays.
- For each Center wavelength value, enter its accompanying **Bandwidth.** This is the filter bandpass width, in nanometers, of the installed filter. For example, the Center/Bandwidth combination of 485/20 will transmit light from 475 to 495 nm (10 nm on either side of the center). The bandwidth is printed on the side of each filter. See the SynergyHT Operator's Manual for details.
- When all values have been entered, click **Send Filters.** The values will be downloaded to the instrument, overwriting its existing wavelength table.

To retrieve filter wheel settings from the instrument:

- Click **Get Filters.** The values will be uploaded to KC4, overwriting the values in this dialog.

Special filter position requirements for the Synergy™ HT with Injectors model:

- For Read & Dispense protocols, KC4 offers an option to block the light between measurements to inhibit photo bleaching.

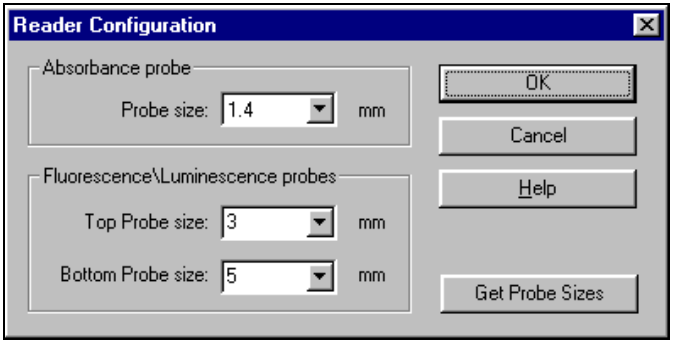
  Single filter set protocols: If light blocking is enabled, a blocking filter (Plug) must be placed in the excitation filter wheel in one of the two positions next to the excitation filter that is specified in the protocol.

  Dual filter set protocols: If light blocking is enabled, two blocking filters (Plugs) must be placed next to each other in the excitation filter wheel.

Reader Configuration

KC4 provides probe size information for some readers via the **Reader Configuration** dialog.

Clarity™ Microplate Luminometer users: KC4's Reader Configuration parameters do not apply to the luminometer.

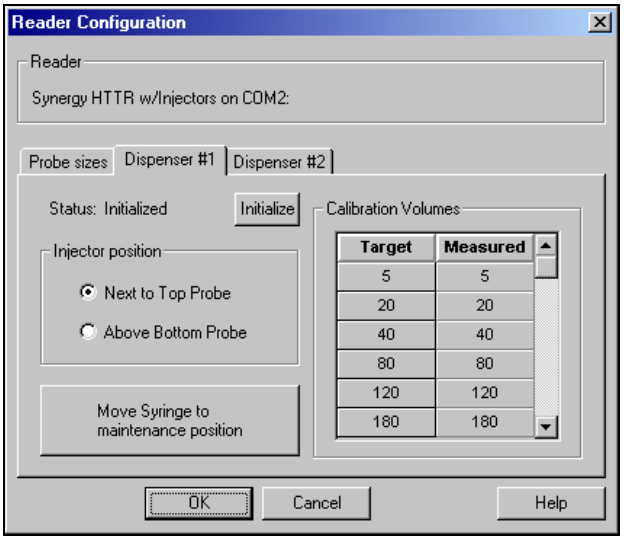


The contents of this dialog vary depending on the currently selected reader. The following parameters/options may be available:

- The **Absorbance Probe** size field displays the diameter of the absorbance probe.
- The **Top Probe size** field displays the diameter of the top probe. If the reader is an FL600™, use the drop-down list to choose the size.
- The **Bottom Probe size** field displays the diameter of the bottom probe. If the reader is an FL600, use the drop-down list to choose the size.
- If supported by the reader, click the **Get Probe Sizes** button to retrieve the size(s) of the probe(s) currently installed in the attached reader.

Dispenser Configuration

For the Synergy™ HT with injectors, KC4 provides access to special configuration functions and settings.



Initializing the Dispenser

If the dispenser module was connected before the reader was turned on, or if a system test was run, the dispenser should initialize automatically. If for any reason the dispenser does not initialize automatically, you can initialize it from KC4. This may be necessary if the module was connected to the reader after the reader was turned on, or following the installation of a new syringe.

To initialize the dispenser:

- In KC4, select **System|Readers,** click the **Configuration** button, and then click the appropriate **Dispenser** tab (#1 or #2).
- Click the **Initialize** button. The syringe drive will move to its home position and its sensors will be verified.

  Upon successful completion, the Status should show "Initialized."

Setting the Injector Position

The instrument dispenses fluid using two injectors. The injector heads can be positioned to dispense fluid from three possible locations: two adjacent to the top probe and one above the bottom probe. The default positioning is one adjacent to the top and one above the bottom.

Moving the injector head from one position to another must be done manually (see the Synergy™ HT Operator's Manual for instructions). After making an adjustment, the new position(s) must be configured in KC4 for recognition by the reader.

To configure the injector positions in KC4:

- In KC4, select **System|Readers,** click the **Configuration** button, and then click the appropriate **Dispenser** tab (#1 or #2).
- Click the radio button corresponding with the current physical injector head position, **Next to Top Probe** or **Above Bottom Probe.**
- Click **OK** to save the change and close the dialog.

Syringe Setup

KC4 provides access to special syringe setup functions for maintenance and calibration purposes.

Important! Do not change the syringe positions or calibrate the dispensers unless instructed to do so as part of installation, upgrade, or maintenance.

Moving a Syringe to its Maintenance Position

If a syringe needs to be installed or replaced, it must first be moved to its Maintenance Position. To do this using KC4:

- In KC4, select **System|Readers,** click the **Configuration** button, and then click the appropriate **Dispenser** tab (#1 or #2).
- Click **Move Syringe to its Maintenance Position.**
- The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected form the drive bracket and unscrewed from the valve (see the Synergy HT Operator's Manual for detailed instructions.)
- Click **OK** to close the dialog.

Controlling Multiple Instruments Simultaneously

If the computer has more than one COM port, more than one instrument can be controlled at a time by running multiple instances of KC4.

Important! Please review the items under "Technical Issues" *before* attempting to control multiple instruments simultaneously.

To control multiple instruments simultaneously:

- Connect the instruments to the computer, one per COM port.
- Launch a single instance of KC4.
- Open System|Readers.
- Select a reader model, define and test its Port settings, then set up its Filter/Wavelengths table. Repeat for each attached reader.
- Once communication is established for all attached readers, launch additional instances of KC4 (one per reader).
- Create, open, and run protocols within each KC4 instance.

Technical Issues

The use of certain functions is restricted when running multiple instances of KC4. These restrictions are in place to maintain integrity with respect to communication, data retrieval, data reduction, and reporting.

When running multiple instances of KC4:

- Individual KC4 instances must control unique reader models. For example, KC4 cannot control two ELx808 instruments at the same time. KC4 *can* control an ELx808 instrument and an ELx808-*I* instrument at the same time.
- Under System|Readers a new Current Reader can be set but its communication and filter/wavelength parameters cannot be changed.
- Software filter/wavelengths tables cannot be updated at any time. For example, clicking the Calibrate button from within the Protocol|Reading dialog generates an error message.
- System|Setup parameters cannot be modified.
- System|Users parameters cannot be modified.
- System|Plate Formats parameters cannot be modified.
- Only one PowerReports document can be edited/printed at a time.

Diagnostics

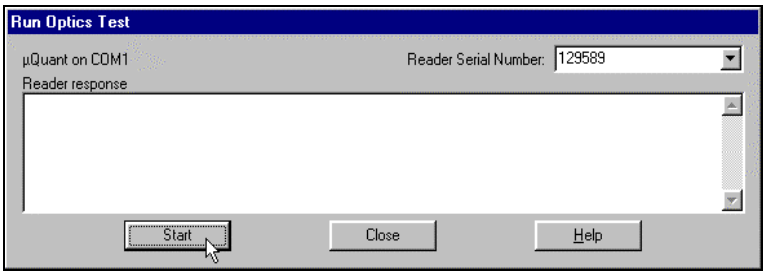
The **Optics Test** and **Universal Plate Test** should be performed routinely to validate and document the consistency of a reader's optical and electronic performance, alignment, accuracy, reproducibility, and linearity.

Optics Test

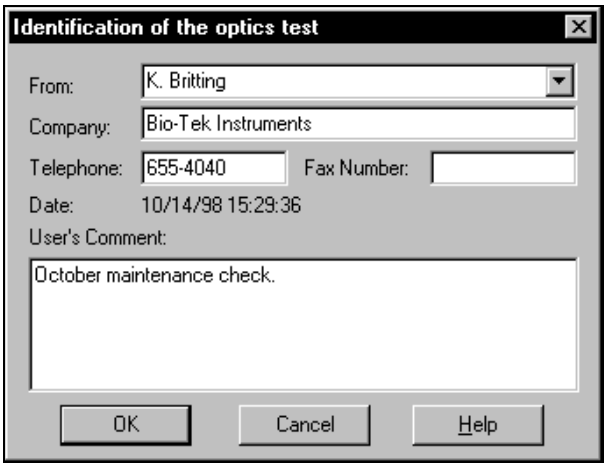
The **Optics Test** (also referred to as the "System Test") validates and documents the consistency of reader optical and electronic performance. For most readers, this test can be performed from within KC4.

To perform an **Optics Test** for all readers except the Clarity™ and the FL600™:

- Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
- Select **System|Diagnostics|Run Optics Test.** The **Run Optics Test** dialog appears.



- Enter the reader serial number, then click **Start** to run the test. When the test is complete, the **Identification** dialog appears:



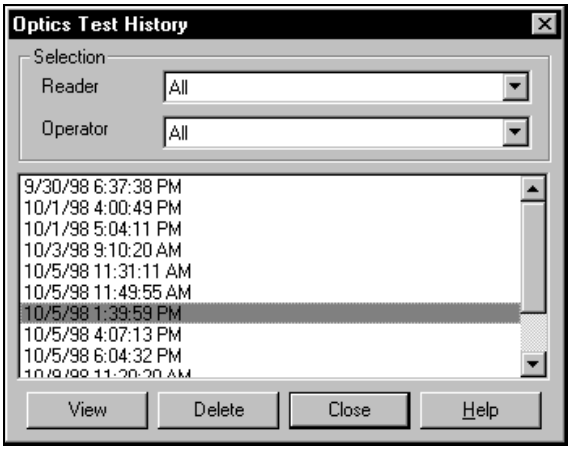
  - Enter the **From, Company, Telephone, Fax Number,** and **Comments** as appropriate.
  - Click **OK** to continue. KC4 displays the results and then saves them in a database in the KC4 folder.

Optics Test History

Following each Optics Test, KC4 saves the test results in a database (CALIB.MDB), located in the KC4 folder.

To view historical Optics Test results:

- Select **Setup|Diagnostics|Optics Test History.** The Optic Test History dialog appears:



  - To view a list of tests for a specific reader, click the **Reader** drop-down arrow.
  - To view a list of tests for a specific operator, click the **Operator** drop-down arrow.
  - To view test results, highlight the desired date/time from the list then click **View.**
  - To permanently delete test results from the database, highlight the desired date/time from the list, then click **Delete.**

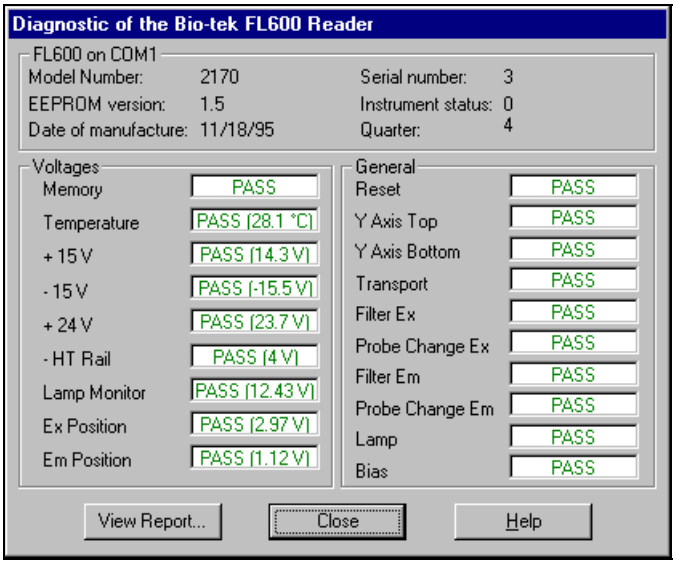
System Test (Clarity)

The System Test for the Clarity™ Microplate Luminometer must be performed using the Clarity PC software. Refer to the Clarity Operator's Manual for instructions.

Optics Test (FL600)

To perform an Optics Test with the FL600™:

- Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
- Select **System|Diagnostics|Optics Test.**
- The FL600 Reader Diagnostics dialog will appear. Click **Start** to run the test.
- As each individual test completes, its pass/fail status appears on the display.
- When the tests are complete, the results will resemble the following:



- Click **View Report** to see a complete listing of commands exchanged between KC4 and the FL600 during the diagnostic procedures. This report can be printed and faxed to Bio-Tek for help with technical support.
- Click **Close** to close the dialog and return to the main display. Test results are automatically saved in a database in the KC4 folder.

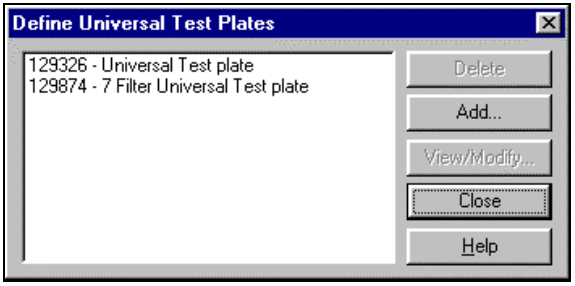
Universal Plate Test

The **Universal Plate Test** uses Bio-Tek's Universal Calibration Test Plate to validate and document the consistency of an absorbance reader's alignment, accuracy, reproducibility, and linearity.

Every Bio-Tek Universal Test Plate comes with a Calibration Test Plate **data sheet.** The data sheet lists the expected absorbance reading for each location in the test plate at several different wavelengths. This data must be entered into KC4 before the Universal Test Plate can be used.

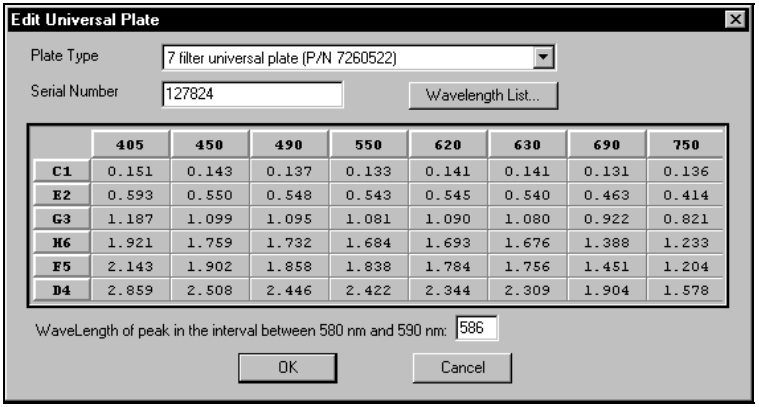
To **define** Universal Test Plates:

- Select **Setup|Diagnostics|Define Universal Plates.** The Define Universal Test Plates dialog appears, listing the currently defined test plates by serial number.



- To modify an existing plate, highlight the desired serial number then click **Modify,** or to add a new plate, click **Add.**

The **Edit Universal Plate** dialog appears:



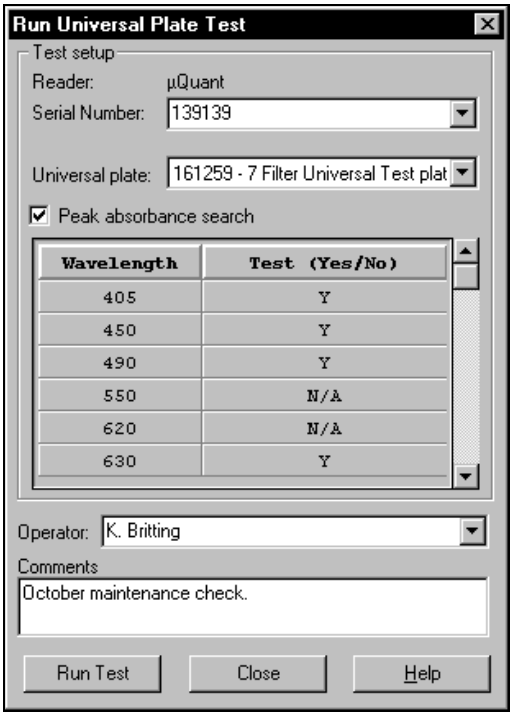
- If this is a new plate, select the **Plate Type** then enter its **Serial Number.**
- The top row of the wavelength grid shows eight unique **wavelengths** for use with the Universal Plate Test. The default wavelengths correspond with the calibration test plate data sheet. To modify one or more of these wavelengths click **Wavelength List.**
- Refer to the calibration data sheet that came with the test plate to determine the expected OD for each test well at each wavelength. For each **Location** (C1, D4, E2, F5, G3, H6), enter the **OD** associated with that well at each wavelength.

  For readers that support spectral scanning, a **Peak Wavelength** value is required. Enter the value in nanometers (nm) **.**
- Click **OK** to save the changes and close the dialog, or **Cancel** to close the dialog without saving changes.

To **run** the Universal Plate Test:

- Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
- The reader should be equipped with the necessary filters to perform the test. Refer to the calibration data table that came with the test plate to determine the required wavelengths.
- Select **System|Diagnostics|Run Universal Plate Test.**

The **Run Universal Plate Test** dialog will appear.



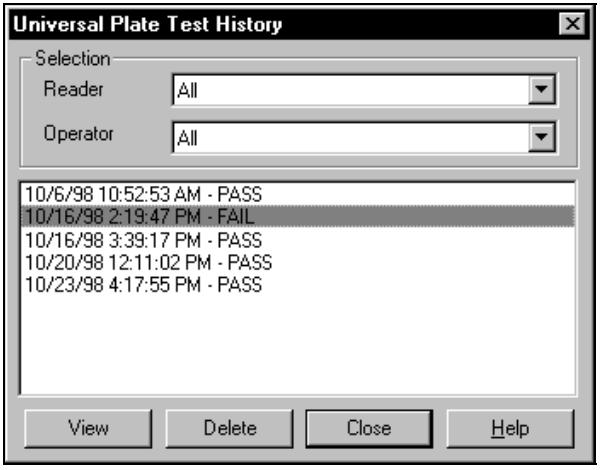
- Enter the **Serial Number** of the reader under test, or click the drop-down arrow to choose an existing number.
- Click the **Universal Plate** drop-down arrow to select the test plate to be used. If the desired test plate is not in the list, select **Setup|Diagnostics|Define Universal Plate** to define a new test plate.
- If supported by the reader, the **Peak Absorbance Search** option is available. If the box is checked, the Peak Absorbance Search is performed in addition to the standard Universal Plate Test.
- Choose the wavelength(s) to be included in the test by clicking the required cells in the **Test (Yes/No)** column. Click in a cell to toggle Yes/No. **Note:** If 'N/A' appears for a wavelength that you want to test, update the reader's filter/wavelength table to include the wavelength.
- Enter the **Operator** name, or click the drop-down arrow to choose an existing name.
- Enter **Comments** pertaining to this plate test (optional).
- Click **Run Test** to begin. Follow the directions provided for loading and turning the test plate. When the test is complete, KC4 displays the results and then saves them in the CALIB.MDB database in the KC4 folder.

Universal Plate Test History

Following each Universal Plate Test, KC4 saves the test results in a database (CALIB.MDB), located in the KC4 folder.

To view historical Universal Plate Test results:

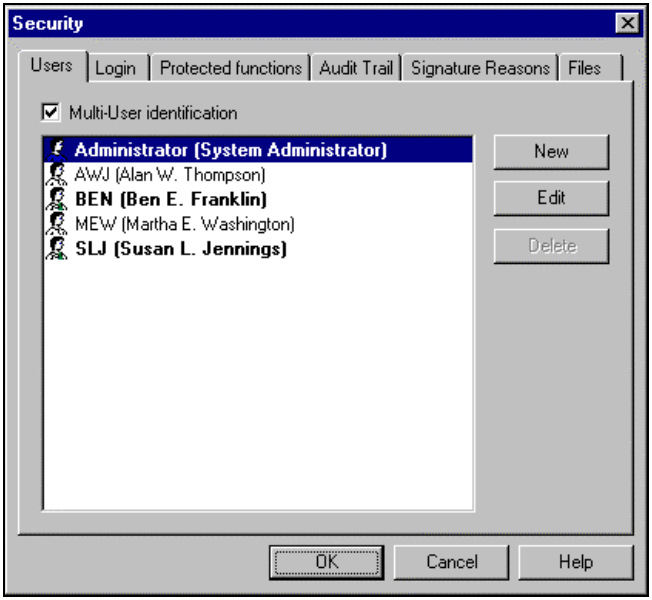
- Select **Setup|Diagnostics|Universal Plate Test History.** The Universal Plate Test History dialog will appear.



  - To view a list of tests for a specific reader, click the **Reader** drop-down arrow.
  - To view a list of tests for a specific operator, click the **Operator** drop-down arrow.
  - To view test results, highlight the desired date/time from the list then click **View.**
  - To permanently delete test results from the database, highlight the desired date/time from the list, then click **Delete.**

Security

KC4 provides several security options to help protect the system and its data from intentional or inadvertent harm or loss. Select **System|Security** to view or edit these options:



Up to six feature tabs are available within the Security dialog. Availability depends on the current KC4 license and whether or not the System Administrator is logged in.

	KC4 v3.4	**KC4 *Signature***
Users	Edit the Administrator's password.	Edit the Administrator's password. Establish and edit individual user accounts and enable or disable Multi-User Identification.
Login	(does not apply)	Enable and define various Login and Password parameters for application in a multi-user environment.
Protected Functions	Determine which KC4 functions shall be protected from use when the Administrator is not logged in.	Determine which KC4 functions shall be protected from use by Standard Users.
Audit Trail	(does not apply)	Enable or disable the appearance of an Audit Trail Notification dialog each time an event is logged to a Data or Protocol Audit Trail, or to the System Audit Trail.
Signature Reasons	(does not apply)	Define a list of terms for users to choose from when signing files, to explain why they are signing.
Files	Select a Preferred Data File format.	Enable or disable the Secured Protocol and Data File Storage feature and select a Preferred Data File format.

User Accounts

*This feature only applies to **KC4 Signature***

A significant component of a secure software environment is the ability to create individual **user accounts** to ensure that only authorized users can gain access to the system and to any restricted functions. KC4 *Signature*'s **Multi-User Identification** feature allows a site-designated System Administrator to create and maintain user accounts.

Multi-User Identification is automatically enabled upon KC4 *Signature* software installation, and it must remain enabled to ensure 21 CFR Part 11 compliance. If, however, you wish to **disable** this feature, select System|Security and uncheck the Multi-User Identification box. This action will cause all user accounts, except the Administrator's, to be permanently deleted.

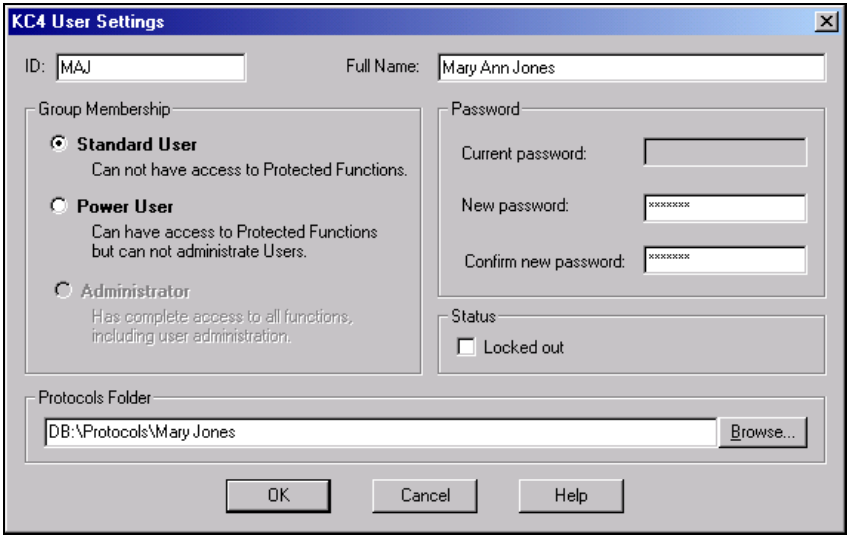
When Multi-User Identification is disabled, users are not required to login to KC4 but the Administrator can still log in and perform the following tasks:

- Change the Administrator's password
- Define system-level protected functions
- Set audit trail notification options
- Enable or disable the Secured Protocol and Data File Storage feature
- Select a Preferred Data File Format

Creating and Modifying User Accounts (Administrator)

To create or modify a user account:

1. Login as the System Administrator.
2. Select **System|Security** and click **New,** or highlight the desired account name and click **Edit.** The KC4 User Settings dialog will appear:

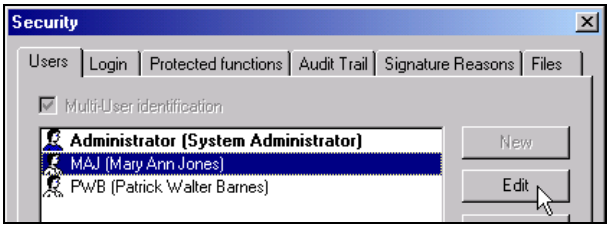


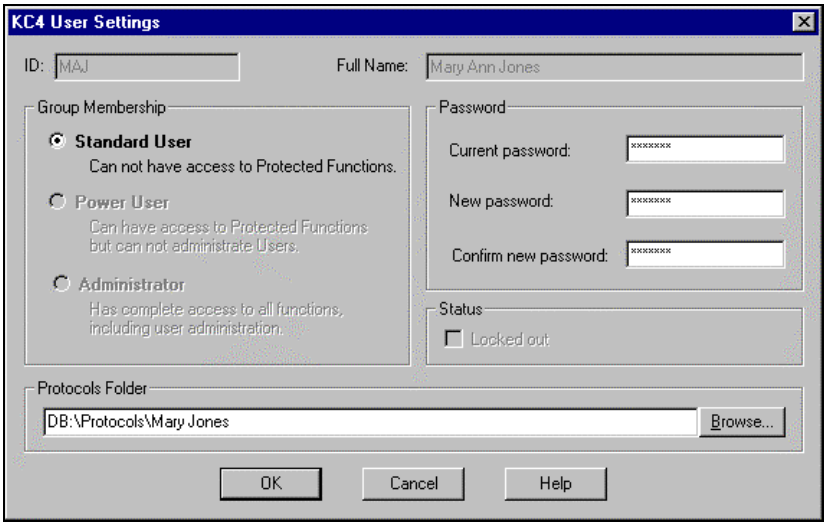
3. Enter a unique **ID** using 1 to 16 alphanumeric characters. The user will enter or select this ID when logging into KC4 and when signing files.
4. Enter the user's **Full Name.** This name will be associated with events logged by this user's actions and with signatures applied by this user.
5. Choose a **Group Membership.**
   - *Standard Users* are denied access to protected functions. (The System Administrator determines which functions shall be protected from use by Standard Users.)
   - *Power Users* are permitted access to protected functions. Power Users cannot administer user accounts or modify Security settings.
6. Enter a temporary **password** for the user in the **New** and **Confirm** fields.
   - Share the password with the user with instructions to change it as soon as possible.
7. (Optional) Specify a folder location from which the user will most often access protocol files. When the user selects Data|New Plate to initiate a plate read, the protocol selection dialog will default to this **Protocols Folder.**
8. Click **OK** to save the changes and return to the Security dialog.

Modifying User Accounts (Standard and Power Users)

Standard Users and Power Users are authorized to modify the Password and Protocols Folder fields within their own accounts. (The ID, Full Name, Group Membership, and Status parameters can only be modified by the System Administrator.)

To modify your user account, begin by logging into KC4 with your user ID and Password. Once logged in, select **System|Security,** highlight your name, and click **Edit.** The **KC4 User Settings** dialog will appear.





To set a new password:

1. Enter your **Current** password (if one exists).
2. Enter a **New** password using up to ten alphanumeric characters (no spaces). Note: If the New password field is inaccessible, the correct Current password must first be entered.
3. Enter the new password again to **Confirm** it.
4. Click **OK** to save the password and close the dialog.
   - If the message "Password minimum length is not respected" appears, this means that the System Administrator has set a minimum password length between 2 and 10 characters and the password you entered is too short.
   - **Important!** The System Administrator cannot retrieve your password if you forget it. If you do forget your password, contact the System Administrator to request a temporary one and then change it immediately.

To specify a Protocols Folder:

1. Using the **Browse** button, select the folder location from which you will most often access protocol files. When you select Data|New Plate to initiate a plate read, the protocol selection dialog will default to the folder defined here. If no folder is specified, KC4 will default to the most recently-accessed folder.
2. Click **OK** to save the change and close the dialog.
   - Specifying a Protocols Folder is optional, and is only available if the 'Secured Protocol and Data File Storage' feature is in use.

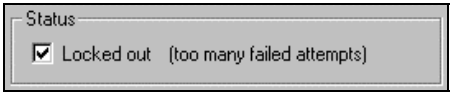
Deleting a User Account (Administrator)

To permanently delete a user account:

1. Login as the System Administrator.
2. Select **System|Audit Trail** to see if the user is currently logged into KC4. You can still delete the account but it will not take effect until the user logs out and tries to log back in, or tries to sign a file.
3. Select **System|Security.**
4. Highlight the desired account name and then click **Delete.** A confirmatory message will appear; click **Yes** to confirm or **No** to cancel the process.

Note: The System Administrator's account cannot be deleted.

Changing an Account's Status (Administrator)



The **Status** box within the KC4 User Settings dialog shows whether or not that user's account is currently locked, and allows the System Administrator to lock or unlock the account. When a user's account is locked, the user cannot log into KC4 and cannot sign files. A user's account may become locked through one of three ways:

- Intentional lock by the Administrator through this dialog
- Automatic lock if the user has exceeded the defined number of successive failed login attempts
- Automatic lock if the user's password has expired

To unlock an account, uncheck the Status box and click OK to return to the Security dialog. Click OK again to return to the main view. The change is effective immediately; the unlocked user is now permitted log into KC4 and to sign files.

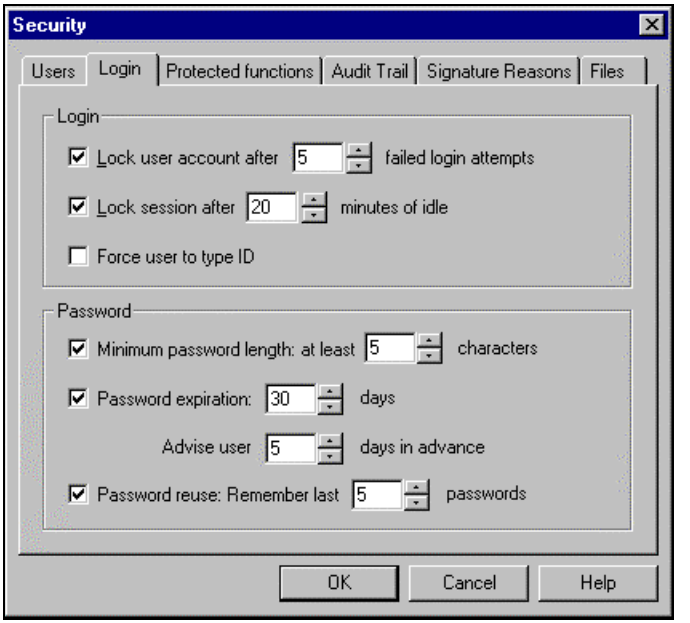
To lock an account, check the Status box and click OK to return to the Security dialog. Click OK again to return to the main view. The change is effective immediately; the locked user is no longer permitted to log into KC4 or to sign files.

- **Note:** If the user is presently logged into another KC4 session, he or she is prevented from signing files but is not automatically logged out.

Login and Password Parameters

*This feature only applies to **KC4 Signature***

Select System|Security, **Login** to access Login and Password parameters. Only the System Administrator is authorized to modify these parameters.



Note: The options shown as enabled above must be enabled to ensure 21 CFR Part 11 compliance. The options' values, however, are site-definable.

To set Login parameters:

1. Enable the desired Login parameters by checking the appropriate boxes.
2. Specify the number of successive **failed login attempts** a user may make before becoming locked out of KC4. This feature only applies to Standard and Power User accounts; the System Administrator's account cannot become locked. Only the System Administrator is permitted to reinstate locked user accounts. Valid entry range: 2 to 10 attempts.
3. Specify the number of minutes that the KC4 session may remain **idle** before it becomes locked and users are required to re-login. A session is considered idle if the user is not operating the keyboard or mouse and if the software is not currently controlling an instrument. Valid entry range: 1 to 1440 minutes.

   - **Note:** For security purposes, KC4 prevents lower-level users from logging in after a higher-level user has been *automatically* logged out of a session (if a user *intentionally* logs out, this restriction does not apply).

     For example, if the Administrator is automatically logged out, only the Administrator is permitted to re-login. If a Power User is automatically logged out, only Power Users and the Administrator are permitted to re-login. If a Standard User is automatically logged out, any valid user may re-login.

4. Enable **Force user to type ID** to require the user to manually type in his or her User ID in the Login and Signature dialogs. Disable this option to permit the user to select his or her User ID from a drop-down list.
5. Click **OK** to save the changes and close this dialog.

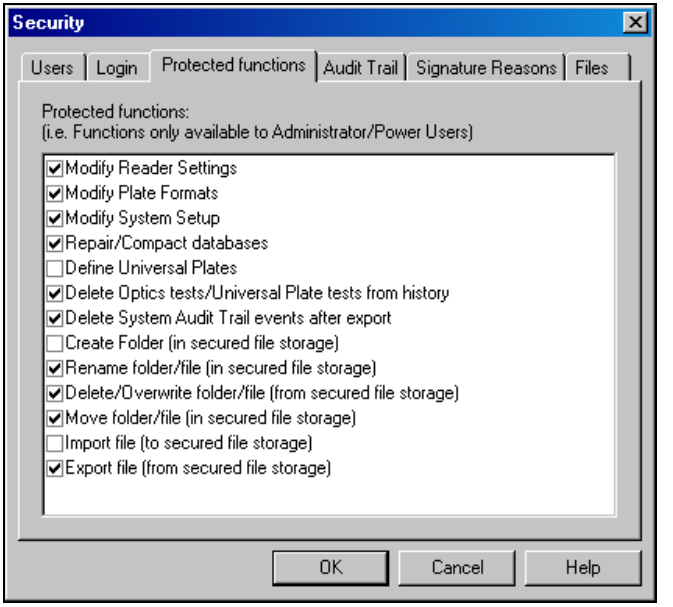
To set Password parameters:

1. Enable the desired Password parameters by checking the appropriate boxes.
2. Specify the **minimum** number of alphanumeric characters required to define a valid password. Valid entry range: 2 to 10 characters.
3. Specify the number of **days** that a password may be used before it KC4 requires the user to change it. If a user allows a password to expire, his or her account will become locked. Only the System Administrator is permitted to reinstate locked user accounts. Valid entry range: 1 to 10000 days.
4. Specify the number of days in **advance** of the password expiration date that KC4 should alert the user to the upcoming expiration. Valid entry range: 1 to the Password Expiration value or 30, whichever is smaller.
5. Specify the number of **unique** passwords that KC4 shall "remember" for each user's account to prevent that user from defining a recently used password. Valid entry range: 2 to 20 passwords.
6. Click **OK** to save the changes and close this dialog.

Protected Functions

Select System|Security, **Protected Functions** to access a list of functions which the System Administrator can protect from use by unauthorized users. **Note:** The picture below shows *all* system-level protectable functions; it may or may not represent your present KC4 configuration.

KC4 *Signature* users: The functions shown as protected below must be protected to ensure 21 CFR Part 11 compliance.



Checked ☑ functions may not be performed by unauthorized users.

- With KC4 v3.4, an unauthorized user is anyone other than the System Administrator.
- With KC4 *Signature*, an unauthorized user is one who is designated as a Standard User.

The following list includes all system-level protectable functions and describes the behavior the unauthorized user can expect from KC4 for each function when it is protected:

- **Modify Reader Settings:** Under System|Readers, the user can change the current reader but cannot alter the Configuration, Port, or Filter/Wavelengths settings. The user will be prevented from changing the current reader or from reading a plate if the action requires an update to KC4's filters/wavelengths table.
- **Modify Plate Formats:** Under System|Plate Formats, the user can view the dimensions of any plate. The user cannot create, modify, or delete plates.
- **Modify System Setup:** Under System|Setup, the user can view the current Protocol Defaults, Formats, Start-Up, and Database settings. The user cannot modify any of these settings.
- **Repair/Compact Databases:** Under System|Setup, Database tab, the user cannot access the Repair/Compact buttons for the KCShared and KCLocal databases.
- **Define Universal Plates:** Under System|Diagnostics|Define Universal Test Plates the user can view the test plate settings but cannot add, modify, or delete a test plate. The user can run a Universal Plate Test.
- **Delete Optics tests/Universal Plate tests from history:** Under System|Diagnostics|Optics Test History and Universal Plate Test History, the user can view historical test results but cannot delete any of them.

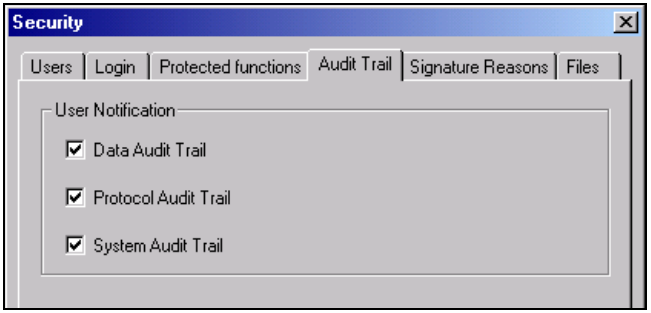
The remaining functions are only found in KC4 Signature:

- **Delete System Audit Trail events after export:** Via the Export button within the System|Audit Trail dialog the user can export audit trail events to a text file but cannot select the 'Delete events after export' option.
- **Create Folder (in secured file storage):** When 'Secured Protocol and Data File Storage' is enabled, the user cannot create new storage folders within the System|Maintain Files, Protocol|Save As, and Data|Save As dialogs.
- **Rename Folder/File (in secured file storage):** When 'Secured Protocol and Data File Storage' is enabled, the user cannot rename folders or files within the System|Maintain Files, Protocol|Save As, and Data|Save As dialogs.
- **Delete/Overwrite Folder/File (from secured file storage):** When 'Secured Protocol and Data File Storage' is enabled, the user cannot delete or overwrite existing folders or files within the System|Maintain Files dialog.
- **Move Folder/File (in secured file storage):** When 'Secured Protocol and Data File Storage' is enabled, the user cannot move folders or files within the System|Maintain Files dialog.
- **Import File (to secure file storage):** When 'Secured Protocol and Data File Storage' is enabled, the user cannot import (paste from clipboard or drag-and-drop from another location) folders or files into the System|Maintain Files dialog.
- **Export File (from secured storage):** When 'Secured Protocol and Data File Storage' is enabled, the user cannot export folders or files out of the System|Maintain Files dialog.

Audit Trail Notification Options

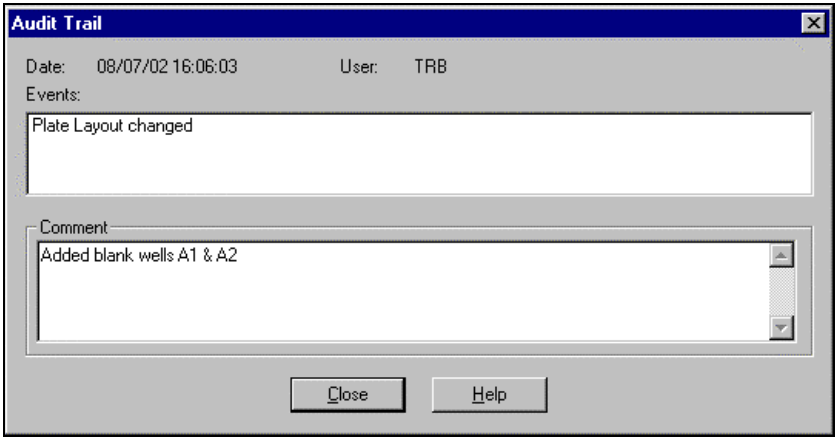
*This feature only applies to **KC4 Signature***

Select System|Security, **Audit Trail** to enable or disable the appearance of an Audit Trail Notification dialog each time an event is logged to the Data, Protocol, or System Audit Trail. Only the System Administrator is authorized to modify these options.

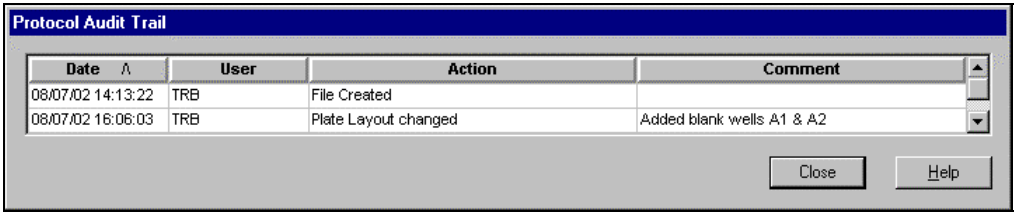


Enabling the **User Notification** options allows users to enter comments for an event. For example, if a user modifies a protocol's Plate Layout, the logged event is simply 'Plate Layout changed.' If the Protocol Audit Trail notification option is enabled, however, the user can provide additional text that will be logged with the event, such as "Added blank wells A1 and A2".

Here is a sample Audit Trail Notification dialog:



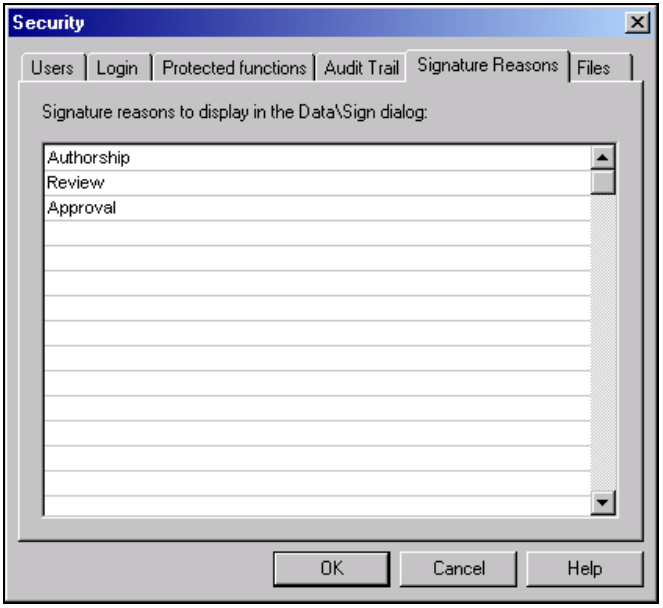
Here is a sample Protocol Audit Trail showing the comments entered above:



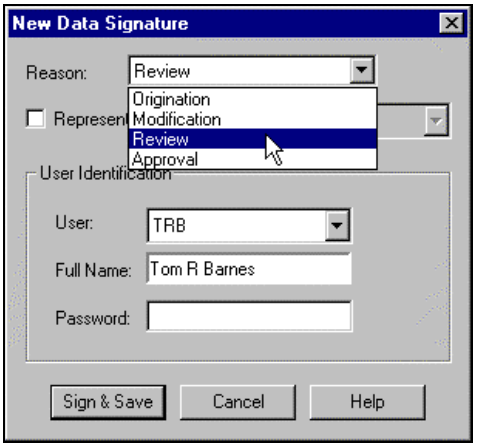
Signature Reasons

*This feature only applies to **KC4 Signature***

Select System|Security, **Signature Reasons** to define a list of terms for users to select from when signing protocols and data files to explain why they are signing. Only the System Administrator is authorized to modify these terms.

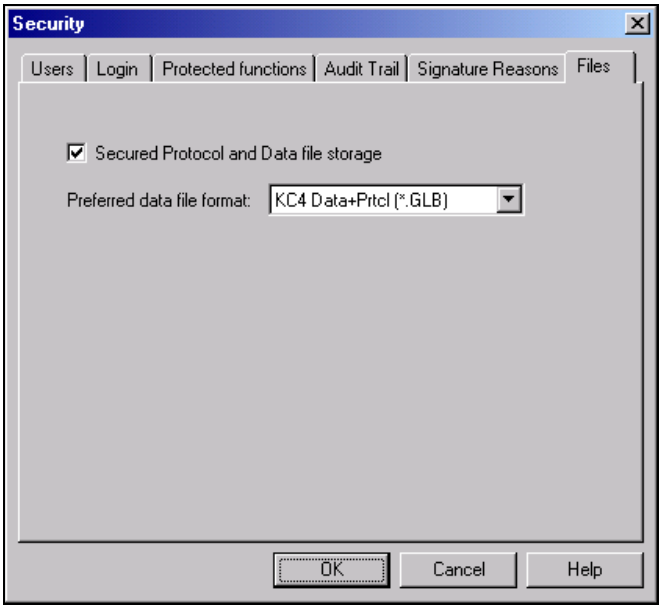


Here is a sample 'New Data Signature' dialog showing the **Reason** drop-down list:



File Storage and Format Options

Select System|Security, **Files** to enable or disable the Secured Protocol and Data File Storage feature and to select a Preferred Data File Format. Only the System Administrator is authorized to modify these parameters.



Secured Protocol and Data File Storage

(KC4 *Signature* only) If this option is enabled, all protocol, plate data, and global data files are stored inside a secure, shared-access database (named KCShared.mdb by default).

- These files can only be accessed from within KC4; they cannot be viewed or otherwise manipulated using separate file management software such as Windows Explorer.
- **Important:** To ensure 21 CFR Part 11 compliance, Secured Protocol and Data File Storage *must* be enabled.
- **Clarity™ Microplate Luminometer users:** Clarity protocol files (with a BPF extension) cannot be stored in KC4's Shared Database. See page 77 for more information.

Preferred Data File Format

When a user chooses to read a plate, the default data file format will be PLA (raw data) or GLB (raw data with protocol parameters), whichever is selected as the **Preferred Data File Format** in this dialog.

- For sites unconcerned with 21 CFR Part 11 requirements, the selection made here is merely a matter of preference (pre-version 3.1 KC4 users may be more comfortable with setting this parameter to .pla).
- For 21 CFR Part 11-compliant sites, setting this parameter to **.glb** will help to streamline the file signing process.

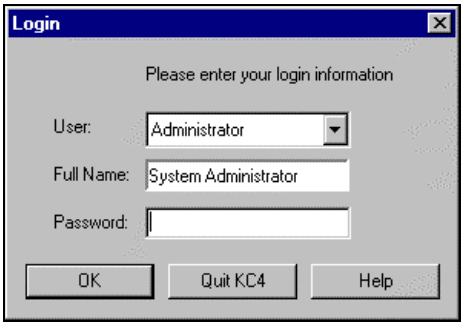
LogIn/LogOut

During typical use with KC4 v3.4, individual users do not log into the software. A System Administrator can optionally log into change the Administrator password, specify protected functions, and specify a preferred data file format.

During typical use with KC4 *Signature*, users **must** log into KC4 to gain access. Each user is identified by a short User ID, a longer Full Name, and a 2 to 10 character Password (see "User Accounts" on page 212 for more information). In addition, the System Administrator logs in to set up and maintain user accounts, and to customize security options.

To log into KC4:

1. Select System|**LogIn** or System|**Administrator LogIn.** The Login dialog will appear:



   - With KC4 *Signature*, the Login dialog also appears each time KC4 is launched.

2. Click the **User** drop-down arrow and select your User ID.
   - If the User field does not have a drop-down arrow, type the ID directly into the field.
3. Type your **Password.** For security purposes it will appear as asterisks on the screen.
4. Click **OK.** KC4 will determine whether or not the User ID / Password combination is valid, and will check to see if your account is "locked" for any reason. If the combination is valid and the account is not locked, KC4's main view will appear.
   - The system may be configured to automatically lock user accounts after a certain number of successive failed login attempts. If your account is locked, contact the System Administrator.

To log out of KC4 v3.4:

1. Select System|**Administrator LogOut.** Control will return to KC4's main view and all unprotected functions will become accessible.

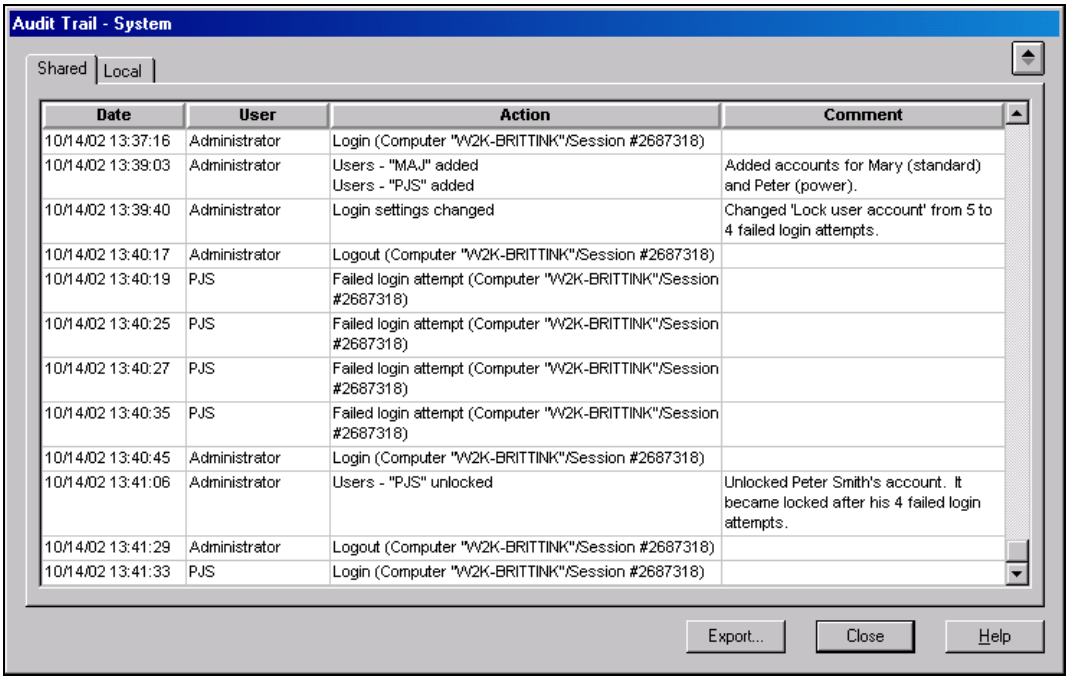
To log out of KC4 *Signature*:

1. Select System|**LogOut.** The Login dialog will appear. KC4's functions will remain inaccessible until the Administrator or another user logs back in.
   - **Recommendation:** To maintain a secure environment, log out any time you are away from the computer. The Login dialog will remain on the screen and you (or another user) can log in at any time. The System Administrator may also set KC4 to automatically log out after a set number of minutes if the system is idle.

Audit Trail

*This feature only applies to **KC4 Signature***

Select **System|Audit Trail** to view a list of all system-level events stored in the shared and local databases currently in use (to see which databases are in use, select System|Setup|Database):



Date	User	Action	Comment
10/14/02 13:37:16	Administrator	Login (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:39:03	Administrator	Users - "MAJ" added Users - "PJS" added	Added accounts for Mary (standard) and Peter (power).
10/14/02 13:39:40	Administrator	Login settings changed	Changed 'Lock user account' from 5 to 4 failed login attempts.
10/14/02 13:40:17	Administrator	Logout (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:40:19	PJS	Failed login attempt (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:40:25	PJS	Failed login attempt (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:40:27	PJS	Failed login attempt (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:40:35	PJS	Failed login attempt (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:40:45	Administrator	Login (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:41:06	Administrator	Users - "PJS" unlocked	Unlocked Peter Smith's account. It became locked after his 4 failed login attempts.
10/14/02 13:41:29	Administrator	Logout (Computer "W2K-BRITTINK"/Session #2687318)	
10/14/02 13:41:33	PJS	Login (Computer "W2K-BRITTINK"/Session #2687318)	

The System Audit Trail displays **Shared** and **Local** events recorded for the following activities:

- User login and logout
- System security updates (user accounts, login and password parameters, protected functions, audit trail notifications, and file location and format)
- Plate format modifications
- Universal test plate modifications
- Reader settings changes (reader type, probe selection, communication parameters, and filters/wavelengths)
- System setup changes (protocol defaults, format settings, start-up options, and database settings)
- User-customizable application settings (such as toolbar and window characteristics)

A note on event sorting under the Shared tab:

The Shared tab displays events recorded by all computers on the network. The date of an event is supplied by the computer on which the event occurs. Because the clocks on different computers are not synchronized, consecutive events on different computers may not be recorded with consecutive dates. Therefore, events under the Shared tab are displayed in the order of their recording in the shared database, not in the order of the event dates.

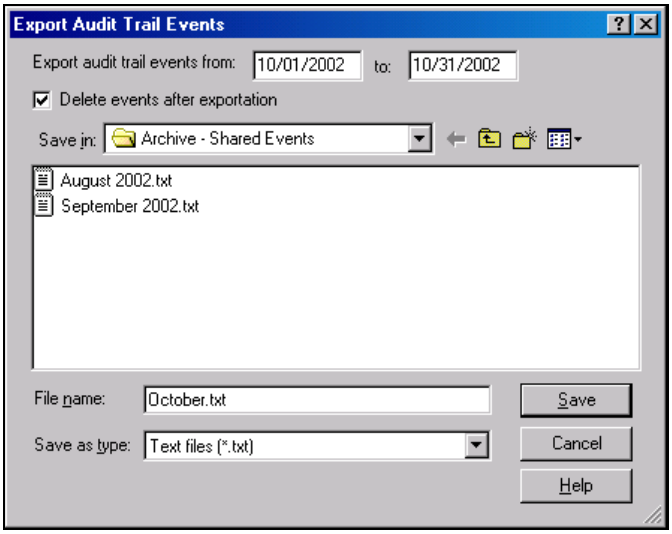
Exporting Audit Trail Events

System audit trail events can be **exported** to a text file and optionally deleted from the System Audit Trail. This feature may be used as part of a site's procedure for archiving past events. Deleting events after export helps to reduce the size of the KCShared and/or KCLocal databases.

Important: When audit trail events are exported to a text file, the text file **does not** reside within KC4's secure database; it resides as a single file in a user-designated location. It is the site's responsibility to ensure the security of exported system audit trail event files.

To export audit trail events:

1. Select System|Audit Trail.
2. Click the **Shared** or **Local** tab.
3. Click **Export.** The Export Audit Trail Events dialog will appear:



4. Enter a valid **date range,** in mm/dd/yyyy format.
5. Select a **destination folder** for the text file.
6. Enter an identifying **file name.**
7. (Optional) Check **Delete events after exportation** to *permanently* remove the events from the audit trail.
8. Click **OK** to export the events.

If events were deleted after export, the System Administrator should do the following to **compact the database:**

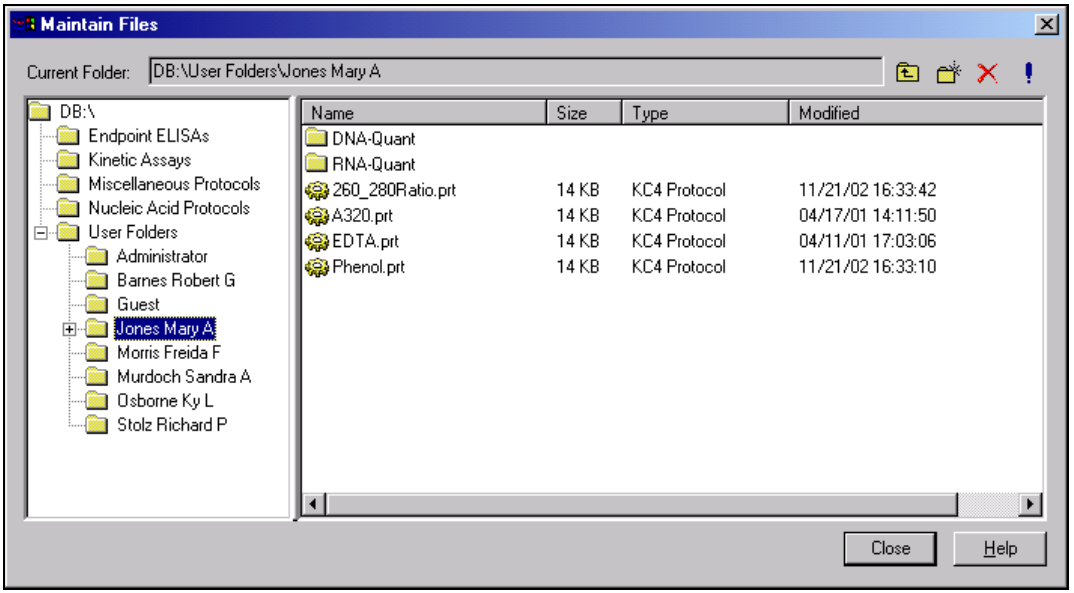
1. Ensure that no KC4 users are currently logged into the shared database.
2. Select **System|Setup, Database.**
3. In the Shared Database or Local Database area as appropriate, click **Repair/Compact,** and then click **OK.** When the process is complete, the database should be smaller in size.

Maintain Files

*This feature only applies to **KC4 Signature***

KC4 *Signature* provides a Windows™ Explorer-style file maintenance utility for working with files stored in the shared database. Authorized users can create folders, move files, delete files, and so on, all within the secure KC4 environment. Events associated with all actions are automatically logged in the System Audit Trail.

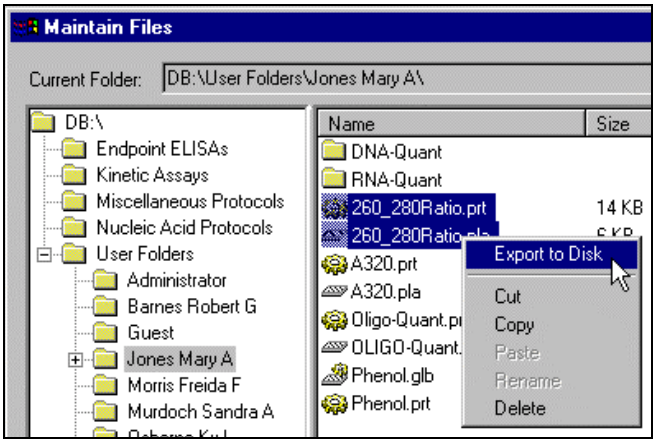
To access the file maintenance utility, select **System|Maintain Files.** All files and folders currently stored in the shared database will be displayed:



- The **Maintain Files** dialog contains two frames. The left frame displays the folder/file tree. The right frame displays the contents of the current folder. All changes are made within the right frame.
- Click to move up one folder level.
- Click to create a new folder.
- Click on a folder to open it and view its contents.
- Highlight a folder or file and click to delete it.
- Click to update the view to show any folders/files that may have been added by another user after you opened this dialog.
- Click the right mouse button to open an editing menu (see "Edit Options" on the next page).

Edit Options

Click the right mouse button to open an **editing menu:**



With the options available through this menu, authorized users can:

- **Export** the highlighted item(s) to a user-designated location outside the shared database.
- Delete (**Cut**) the highlighted item(s) and move them to the clipboard.
- **Copy** the highlighted item(s) to the clipboard without deleting them.
- **Paste** (or import) folders and files from the clipboard into the current folder.
- **Rename** the highlighted folder or file.
- **Delete** the highlighted item(s) without first moving them to the clipboard.

Exporting Files

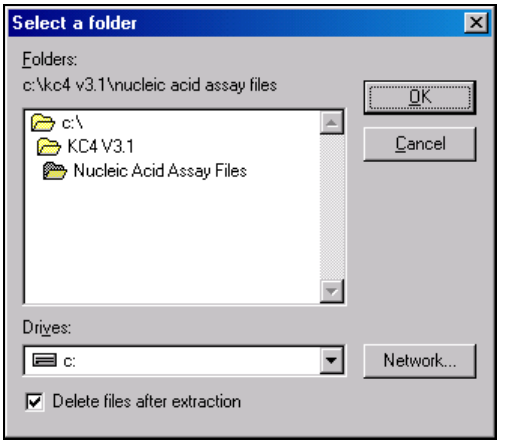
Protocol, plate data, and global data files residing inside the shared database can be exported out of the database through the Maintain Files dialog.

Important: When files are exported out of KC4's secured database, they will reside on a disk or the network in a user-designated location. It is the site's responsibility to ensure the security of exported files.

To export a file:

1. Select **System|Maintain Files.** Locate the desired file.
2. Highlight the file, click the right mouse button, and select **Export to Disk.**
   - The System Administrator can **protect** the use of the 'Export' and 'Delete Files After Extraction' functions. If you are trying without success to export and/or delete files, you may not be authorized to do so.

The **Select a Folder** dialog will appear.



3. Select the target **Drive** and **Folder** (either the local hard drive or a network drive).
4. (Optional) Check **Delete files after extraction** to *permanently* remove files from the database after they are exported.
5. Click **OK** to continue.
   - Files are exported in their proprietary formats. Files can be opened by a KC4 program that does not use the shared database, or they can be imported (pasted from the clipboard) into another shared database.

If files were deleted after export, the System Administrator should do the following to **compact the database:**

1. Ensure that no KC4 users are currently logged into the shared database.
2. Select **System|Setup, Database.**
3. In the Shared Database area, click **Repair/Compact,** and then click **OK.** When the process is complete, the database should be smaller in size.

Importing Files

Protocol, plate data, and global data files residing outside the shared database can be imported into the database through the Maintain Files dialog. This includes any unsaved files that were automatically saved to a temporary location following a database connection failure.

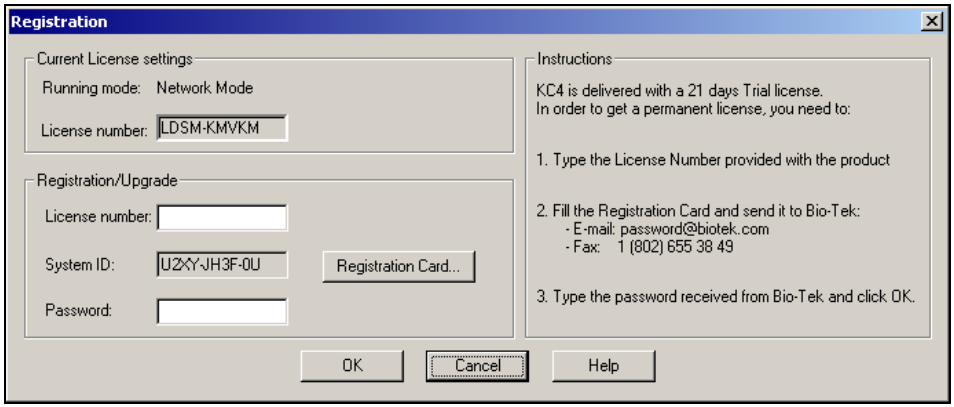
To import a file:

1. Select **System|Maintain Files.** Locate and make current the folder in which the imported file will reside.
2. Launch Windows Explorer and locate the desired file.
3. Highlight the file, click the right mouse button, and select **Copy.**
4. Switch back to KC4. Place the cursor inside the Maintain Files dialog, click the right mouse button, and select **Paste.**
   - The System Administrator can **protect** the use of the Import function. If you are trying without success to import files, you may not be authorized to do so.

Registration/Upgrade

Twenty one (21) days after installation (when the Trial mode is over), your KC4 software will switch to Demonstration mode unless you enter a valid password. To apply for and enter a password:

1. Select **System|Registration.** The Registration dialog will appear:


Registration
Current License settings
Running mode: Network Mode
License number: LDSM-KMVKM
Registration/Upgrade
License number:
System ID: U2XY-JH3F-0U
Registration Card...
Password:
Instructions
KC4 is delivered with a 21 days Trial license.
In order to get a permanent license, you need to:
1. Type the License Number provided with the product
2. Fill the Registration Card and send it to Bio-Tek:
- E-mail: password@biotek.com
- Fax: 1 (802) 655 38 49
3. Type the password received from Bio-Tek and click OK.
OK
Cancel
Help

2. If the **License Number** field is empty, enter the license number provided with your KC4 software package.
3. Click the **Registration Card** to verify the license number and then open a form. Enter the required information and send the form to Bio-Tek via fax or email.
4. Bio-Tek will respond with a password via fax or email. When you receive your password from Bio-Tek, enter it in the **Password** field and click **OK.**

KC4 Reinstallation

If you are reinstalling KC4, you must fill out and submit a **Reinstallation Form** to obtain another password. Reinstallation is defined as:

- Re-installing a KC4 version on the same PC (for example, after a hard disk replacement).
- Un-installing a KC4 version from one PC and installing the same version on another PC.

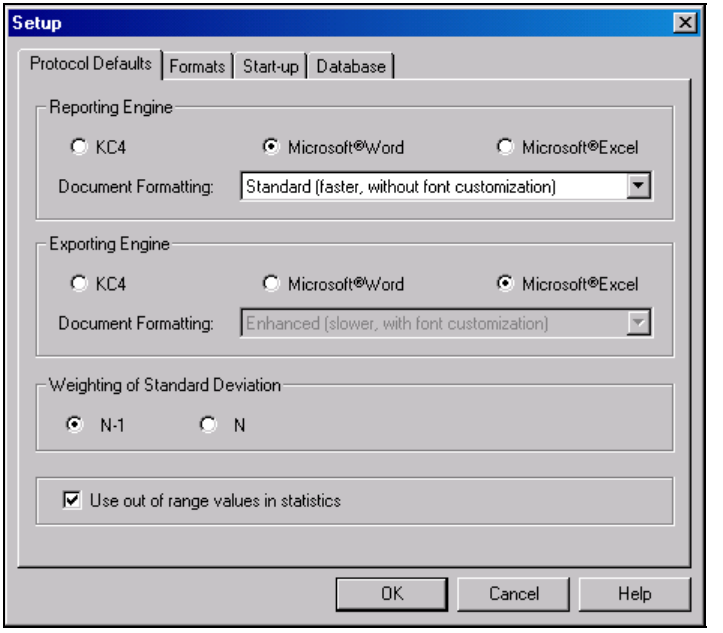
A Reinstallation Form (.doc and .txt formats) complete with instructions can be found in the root KC4 folder.

Setup

Select **System|Setup** to set various global parameters, including reporting and exporting engines, import/export separator, and startup options.

Protocol Defaults

Select **System|Setup|Protocol Defaults** to specify several default settings for new protocols:



- Select the default **Reporting** and **Exporting Engines.**

  With KC4's standard reporting and exporting engines you can customize the contents (and to some extent the appearance) of printed reports and exported plate data. *All* KC4 users have access to these standard functions. Reporting and exporting with user-designed Microsoft® Excel 2000 and Word 2000 documents is supported with KC4's PowerReports feature. For more information on the Word/Excel reporting options, see *PowerReports* near the end of this manual.

  If Word was chosen as the reporting and/or exporting engine, choose a **Document Formatting** option of Standard or Enhanced to tell KC4 how to prepare documents for printing and print previewing. The default selection is Standard.

  - If **Standard** is selected, document formatting is faster because KC4 does *not* apply any custom font characteristics from the Plate Layout or Cutoffs dialogs. Well IDs and cutoff symbols are printed using just the default font characteristics.
  - If **Enhanced** is selected, document formatting is slower because KC4 does apply custom font characteristics to Well IDs and cutoff symbols.

- Select the **Weighting of Standard Deviation:**

  N-1 Used in calculations where the data is assumed to represent a sample from a larger population.

  N Used in calculations where the data is assumed to represent the total population from which the sample was taken.

- Choose whether or not to **use out of range values in statistics.** Out of range values are those values reported as '<xxxx' or as '>xxxx'.

  - **Example:** SPL1 has three replicates reported with the following concentrations:

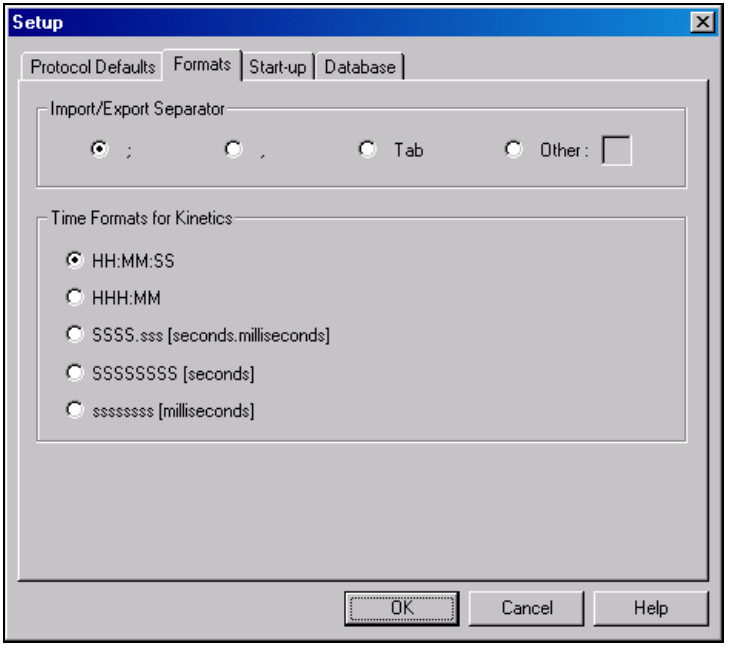
    well A1 : 10.230
    well A2 : <10 (out of range value)
    well A3 : 10.450

    If the option is *not* selected, the software will not use the concentration of well A2 in the statistic calculations. Therefore the mean, standard deviation and CV of SPL1 will be 10.34, 0.155, and 1.5%, respectively. If the option *is* selected, concentration of well A2 is taken into account (the value of 10 is used in the statistic calculations). Therefore the mean, standard deviation and CV of SPL1 will be reported as <10.22, 0.225, and 2.2%, respectively.

    Important! Exercise caution when enabling this option, as it can lead to biased results particularly for standard deviation and CV. As a warning, statistic results arising out of the use of this option are displayed in italic in the report.

Formats

Select **System|Setup|Formats** to choose an import/export separator and a time format for kinetics.



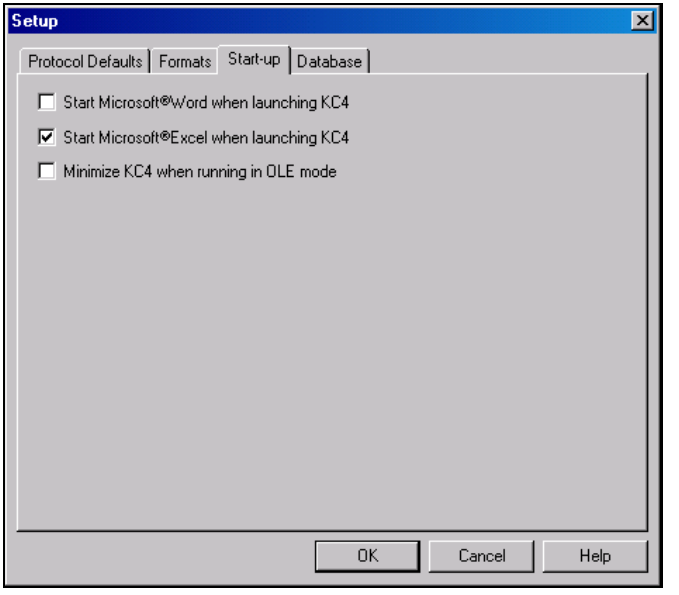
- Select an **Import/Export Separator** (delimiter) for use in export file formats. Choose semi-colon, comma, or tab, or specify a custom separator (such as |) in the **Other** field.
  - Before **importing** a plate data file, ensure that the selected Import/Export Separator matches what is used in the file.
- KC4 offers the following **Time Formats for Kinetics:**

HH:MM:SS	Average duration kinetics
HHH.MM	Long duration kinetics
SSSS.sss	Average duration kinetics, reported in seconds and milliseconds
SSSSSSSS	Average duration kinetics, reported in seconds
sssssssss	Fast kinetics, reported in milliseconds

  - When involved in calculations such as Transformations, Cutoffs and Validation conditions, time values are always expressed in milliseconds, regardless of the time formats chosen in the **System|Setup** menu. If you need to use other units, define a single-plate transformation to make the conversion.
  - When time values are used on the Y axis of a Standard Curve, they are always expressed in seconds.

Start-Up

Select **System|Setup|Start-Up** specify behavior each time KC4 is launched.



- Specify whether or not to **start Word** or **Excel when launching KC4.** Keeping these applications open saves time when creating new report and export templates; you won't have to wait for them to load each time you make a change.
- If you are running KC4 with an external robotic application (such as Bio-Tek's Bio-Stack PC Control software), click **Minimize KC4 when running in OLE mode.** When KC4 starts it will automatically minimize, permitting access to the external application.

Database

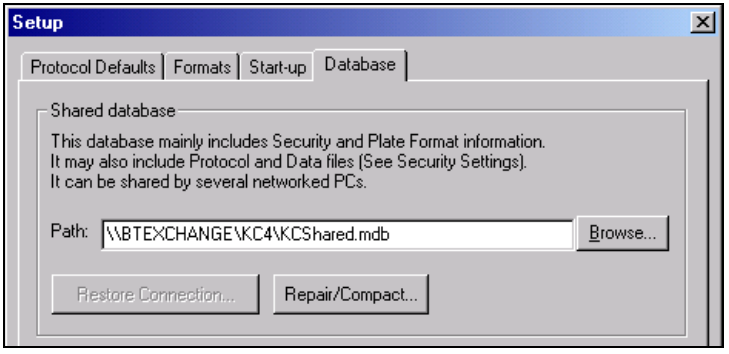
KC4 uses three different databases for storing information. These databases are secure, meaning that they can only be opened and updated through KC4.

- **KCShared.mdb** contains security and plate format information. With KC4 *Signature*, this database also contains user accounts; protocol, plate data, and global data files and their associated audit trails; and a system audit trail for shared events. Following installation, KCShared.mdb is stored on the computer's hard drive but it can be moved to a network location for secure storage and multi-user access. This database may also be copied and renamed (if, for example, your site requires a separate shared database for each project).
- **KCLocal.mdb** contains all of the settings defined by the user under System|Readers and System|Setup. With KC4 *Signature*, this database also contains an audit trail for local events. KCLocal.mdb is stored on the computer's hard drive, and it cannot be moved or renamed.
- **CALIB.mdb** contains Optics and Universal Plate Test setup values and saved test results. CALIB.mdb is stored in the root KC4 folder. It cannot be moved or renamed, and there are no special functions associated with it.

Select **System|Setup|Database** to access information and functions related to KC4's shared and local databases.

Shared Database

The **Shared Database** area contains the current location and name of the shared database, a Restore Connection button, and a Repair/Compact button.



The **Path** setting must be changed if the location and/or the name of the KCShared.mdb file changes. For example, following KC4 installation the System Administrator is advised to make a copy of the installed KCShared.mdb file and store the copy on a shared location on the network. To gain access to this shared file, the Path setting must change to reference the network location.

- Before changing the Path, a KCShared.mdb file must already exist in the new location; this feature does not create a new KCShared.mdb file.
- The shared database name is not *required* to be **KCShared**.mdb. If your site requires the use of multiple shared databases (one per project, for example), the file can be renamed via Windows Explorer. The .mdb extension IS required.

- **Tip:** With some operating systems, KCShared.mdb is installed in the **Application Data** folder under \Documents and Settings\All Users. If you are looking for the Application Data folder on your computer and can't find it, it is probably hidden. To reveal this folder, open Microsoft Explorer and select Tools|Folder Options|View. Make sure that 'Show hidden files and folders' is enabled.

To change the shared database Path:

1. Click **Browse.** A standard file open dialog will appear.
2. Locate the desired shared database file and click **Open.** Control will return to the Database dialog and the Path field will fill in with the new name.
3. Click **OK.** A confirmatory message will appear, stating that the database change will not take effect until KC4 is closed down and relaunched.
   - "Modify system setup" is a protectable function. If you have tried without success to change the Path, you may not be authorized to do so. Contact your KC4 System Administrator.
4. Click **Yes** to close KC4.
5. **Multi-user sites:** Repeat these steps for all computers that use the shared database.

The **Restore Connection** button becomes active after a critical database error is detected (if the database path did not change). This button must be clicked after a critical database error occurs. For example, if the network connection drops while KC4 is open, a critical database error can occur because the shared database is no longer accessible. When the network connection is restored, click Restore Connection to reconnect KC4 with the database.

It may be necessary to click the Restore Connection button several times to successfully restore the database connection. If this message appears:

- Click **Retry** to try to restore the database connection again.
- Click **Cancel** to abort the process and regain control of KC4.
- Click **Quit KC4.** Unsaved files will be saved as temporary files on the computer's hard drive; the pathname will be provided. These files can be renamed and moved using Windows Explorer and optionally imported into the shared database.

The **Repair/Compact** button should be used following these events:

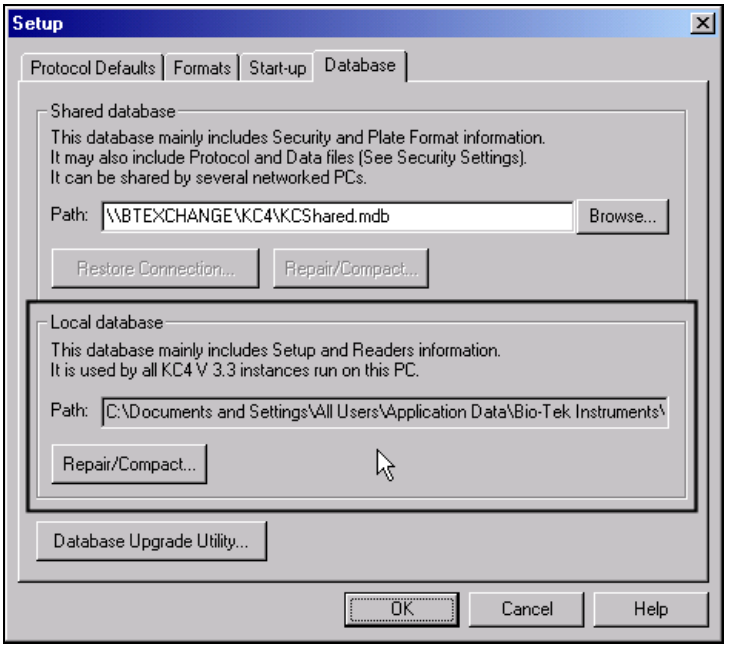
- After reconnecting to the shared database following a database error, in case the database has been corrupted.
- (KC4 *Signature*) After System Audit Trail events are exported *and deleted*, to reduce the size of the database.

- (KC4 *Signature*) After files are exported *and deleted* from the Maintain Files dialog, to reduce the size of the database.
- **Note:** Repair/Compact may only be performed if no other KC4 session is using this file.

When Repair/Compact is clicked, KC4 performs a safety backup of the database in its present location (KCShared_yymmdd_hhmmss.mdb) before beginning the repair/compact procedure.

Local Database

The **Local Database** area of the Setup|Database dialog contains the current location of KCLocal.mdb and a Repair/Compact button.



The **Path** setting for KCLocal.mdb cannot be changed. If the pathname is larger than the width of the field, click once in the field and press the right arrow button on your keyboard to scroll to the right. Knowing where KCLocal.mdb is stored is useful if your site requires a periodic back up of this file.

- **Tip:** With some operating systems, KCLocal.mdb is installed in the **Application Data** folder under \Documents and Settings\All Users. If you are looking for the Application Data folder on your computer and can't find it, it is probably hidden. To reveal this folder, open Microsoft Explorer and select Tools|Folder Options|View. Make sure that 'Show hidden files and folders' is enabled.

The **Repair/Compact** button should be used following a KCLocal.mdb database error. When the button is clicked, KC4 performs a safety backup of the database in its present location (KCLocal_ yymmdd_hhmmss.mdb) before beginning the repair/compact procedure.

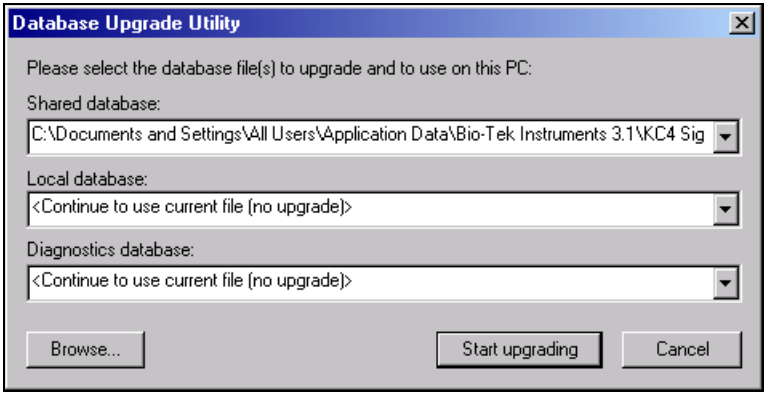
Database Upgrade Utility

Use the **Database Upgrade Utility** to update Shared, Local, and Calibration databases created with earlier versions of KC4 so that they will be compatible with the current version of KC4. For example, if you are upgrading from KC4 version 3.1 to 3.4, your version 3.1 databases must be converted before they can be used by KC4 version 3.4. This utility runs automatically during installation. It can also be run manually from the System|Setup, Database dialog.

To run the Database Upgrade Utility:

1. If possible, identify the locations and names of all databases to be upgraded.
2. Close all other instances of KC4 running on the PC.
3. If upgrading a database located on a network drive, make sure that no other KC4 users are currently accessing that database.
4. Close any open, unsaved KC4 files.
5. Select **System|Setup** and click the **Database** tab.
6. Click the **Database Upgrade Utility** button.
   - KC4 automatically begins a search of the fixed, non-removable drives (e.g., diskette, ZIP, JAZ, and network drives are not automatically searched). KC4 searches for the following files: KCLocal.mdb, KC*.mdb, and Calib.mdb. **Note:** This automatic search will not find Shared database files if their names do not begin with "KC".
   - If you know the names and locations of the databases you wish to upgrade, and/or if the databases reside on a drive that is not automatically searched, click the **Stop** button.

When the search is complete, or when the Stop button is clicked, the Database Upgrade Utility dialog appears:

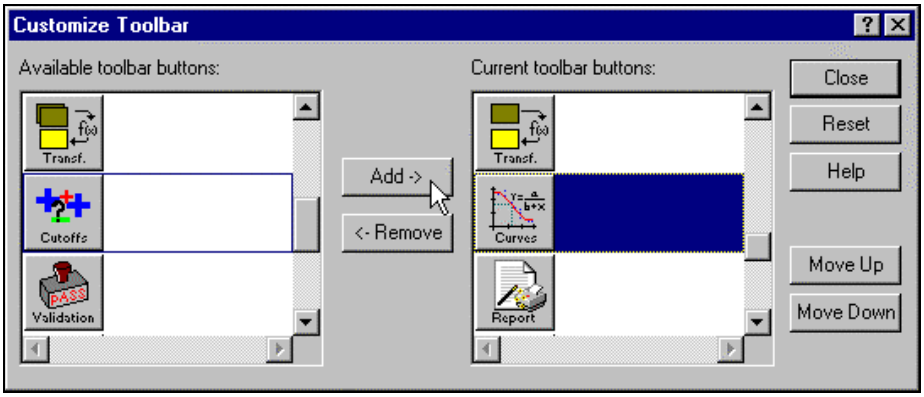


- Click the drop-down arrows to see which databases KC4 found. Click on the desired database name to select it for upgrading.
- Click the **Browse** button to search for a database. When the database is located, highlight it and click Open. KC4 will insert the name in the appropriate field (Shared, Local, or Diagnostics).
- Click **Start Upgrading** to begin the process. When prompted, enter the name(s) for the converted databases. (The original databases will remain unchanged).
- When the process is complete, restart KC4. The upgraded database(s) will be in use.

Toolbar

The **Toolbar** contains buttons that, when clicked, allow quick access to KC4 functions. Accessibility of a particular toolbar button depends on whether or not its function may be used in the current environment. For example, the Biograph button is accessible only if results are displayed and a curve has been generated.

When KC4 is installed, the toolbar contains a set of default buttons. These buttons can be customized by selecting **System|Toolbar.**



- To add a button to the toolbar, highlight the desired button under Available Toolbar Buttons, then click **Add.**
- To remove a button from the toolbar, highlight the button under Current Toolbar Buttons, then click **Remove.**
- To change the position of a button in the Current toolbar list, highlight it then click **Move Up** or **Move Down.**
- To restore the toolbar to its default buttons, click **Reset.**
- To save the changes and update the toolbar, click **Close.**

On most displays, the toolbar can easily display up to 16 icons. All possible icons are shown and described below and on the following pages:

Create a protocol using the **Protocol Wizard.** This is offered as an alternative to using the Protocol Menu. (Protocol|Wizard)

Initialize memory for a **New** data file. Click this button before reading a plate. (Data|New)

Read a plate (or enter data manually, or read data from a file). Before clicking this button, ensure that the reader is connected to the computer, and that the communication parameters are properly configured. (Data|Read Plate)

Save a data file. Plate data files (raw data) use the extension *.PLA. Global data files (raw data + protocol parameters) use the extension .GLB. Selection of the target directory is possible using the Browse facility. (Data|Save)

Sign a data file. In a multi-user system, global data files (raw data + protocol parameters) can be electronically signed. (Data|Sign)

Print the results report. The appearance of the button depends on the Report Engine setting under Protocol|Options (Excel, Word, or KC4). Report templates are created under Protocol|Report. (Data|Print)

Preview the results report before printing. The appearance of the button depends on the Report Engine setting under Protocol|Options (Excel, Word, or KC4). Report templates are created under Protocol|Report. (Data|Print Preview)

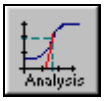
Export the plate data to a file. The appearance of the button depends on the Export Engine setting under Protocol|Options (Excel, Word, or KC4). Export templates are created under Protocol|Export. (Data|Export)

Enter **Sample IDs** associated with the plate. This can be done before a plate is read, or after a plate read when results are displayed in KC4. (Data|Samples)

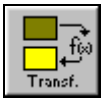
Define the text to replace the **prompts** in the Data|Plate Description and Data|Read Plate dialogs. (Protocol|Plate Description)

Define or modify reading parameter **settings.** If this button is inaccessible, select Protocol|New Protocol to select a protocol type, then click Settings to define the reading parameters. (Protocol|Reading)

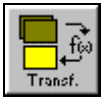
Configure the plate **Layout** by assigning a meaningful identifier to each well. Typical identifiers include “BLK”, “STD”, and “SPL”. Standard concentrations and dilutions are also defined here. (Protocol|Plate Layout)



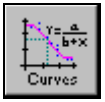
For kinetic, spectrum, or scanning protocols, define related **Analysis** parameters for data reduction. (Protocol|<type> Analysis)



Define or modify **Single Plate Transformations** to perform calculations within the same plate/data set. Typical uses include using specific wells as local blanks for other wells and calculating ratios between wells. (Protocol|Single Plate Transformations)



Define or modify **Multi Plate Transformations** to perform calculations utilizing more than one plate/data set. For example, create a multi plate transformation to subtract the 630 nm measurement values from the 405 nm measurement values in all wells. (Protocol|Multi Plate Transformations)



Define or modify **Curve** fitting parameters. Configurable parameters include Data to Interpolate, Curve Fit Method, and Interpolation values. This button is available if well identifiers with concentrations or dilutions are defined in the Plate Layout. (Protocol|Curve)

Define up to five sets of **Validation** criteria to assess the suitability of select wells. If the criteria are not met, KC4 generates a warning to indicate that results are suspect. (Protocol|Validation)

Define **Cutoffs** to screen selected data into defined categories based on fixed or variable threshold limits. During data reduction, results are evaluated against the cutoff criteria and each well is assigned a user-defined symbol. (Protocol|Cutoffs)

Create a **report** template. The appearance of the button depends on the Report Engine setting under Protocol|Options (Excel, Word, or KC4). The template is stored with the protocol when it is saved. (Protocol|Report)

Create an **export** template. The appearance of the button depends on the Export Engine setting under Protocol|Options (Excel, Word, or KC4). The template is stored with the protocol when it is saved. (Protocol|Export)

Define various protocol **options** including the report and export engines, and automatic save, append, report, and export procedures. (Protocol|Options)

Specify which functions in the currently open protocol shall be **protected** from use by unauthorized users. (Protocol|Security)

Open the **Maintain Files** dialog to create folders, move files, delete files, and so on, within the shared database. (System|Maintain Files)

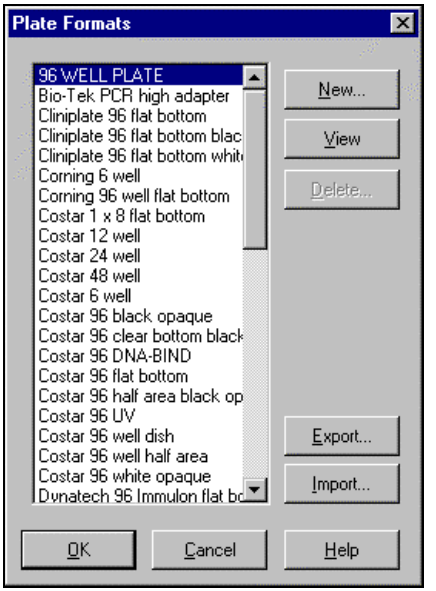
Launch the **Biograph** application to view the calculated curve(s). This button is accessible only if results are displayed and a curve has been successfully generated. (Display|Curve)

Control the attached reader's door/carrier movement or incubation. This button is accessible only if these functions are supported by the current reader. (System|Reader Control)

Plate Format

This dialog lists all of the plate types currently stored in the shared database. For each plate type, the database contains formatting information such as width, length, height, and the number of rows and columns. When a plate read is initiated, KC4 checks to see which plate type is selected in the Reading Parameters dialog and then sends the appropriate formatting information to the reader. The reader uses this information to precisely position the plate when taking measurements. Not all readers support all of the plate formats in this list. Consult your reader's operator's manual for a list of supported plate sizes.

KC4 comes with format information for more than forty industry-standard microplates. You can add your own microplates by selecting **System|Plate Formats:**



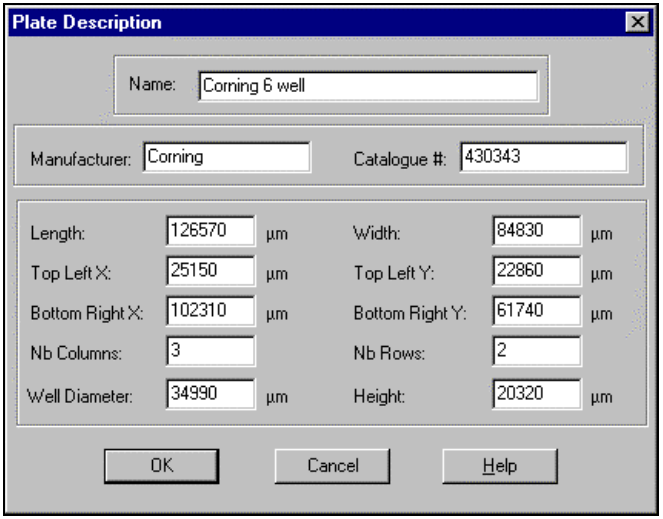
The **Plate Formats** dialog lists the currently defined plate types.

- To add a new plate, click **New.**
- To modify a plate, highlight the plate name and click **Modify.**

  Warning! Modifying the dimensions of the installed plate types is not recommended; consider adding a new plate type instead. Please contact Bio-Tek with any questions regarding the current dimensions.
- To view the format information for any plate, highlight the plate name and click **View.**
- To delete a plate, highlight the plate name and click **Delete.**
- To export plate format information to a text file, click **Export.** The export function is useful if you wish to add custom plate formats from earlier KC4 versions. See "Exporting/Importing Plate Formats" for more information.
- To import the contents of an external plate database file (either a .txt or a .pld file), click **Import.** The import function is useful if you wish to work with custom plate formats created with an earlier KC4 version. See "Exporting/Importing Plate Formats" for more information.

Plate Description

When creating or modifying a plate, information is entered in the **Plate Description** dialog:



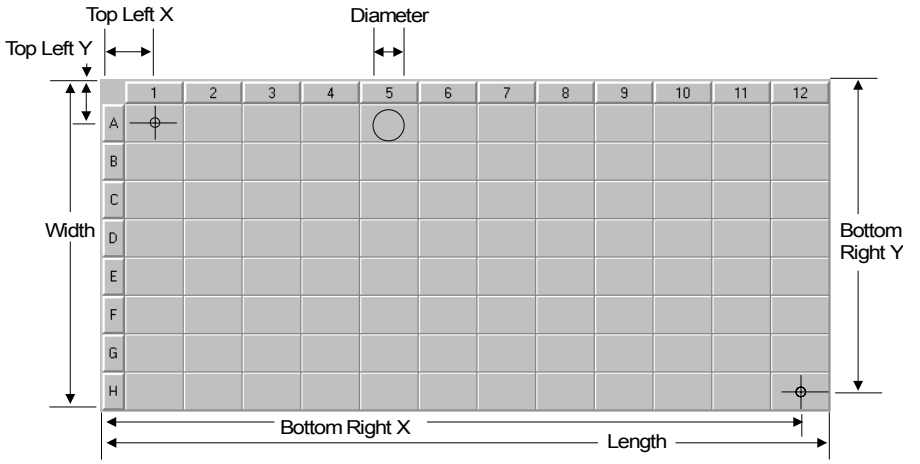
- **Name** stores descriptive, unique text appropriate for the microplate. This name appears in the Plate Type selection list within the Reading Parameters dialog.
- **Manufacturer** stores the name of the microplate manufacturer.
- **Catalogue #** stores the microplate identification number.
- **Length** is the longest dimension of the plasticware (the x axis).
- **Width** is the shorter dimension of the plasticware (the y axis).
- **Top Left X** is the distance from the left side of the plate to the center of well A1.
- **Top Left Y** is the distance from the top of the plate to the center of well A1.
- **Bottom Right X** is the distance from the left side of the plate to the center of the last well on the plate. In the case of a 96-well plate, this would be H12.
- **Bottom Right Y** is the distance from the top of the plate to the center of the last well on the plate. In the case of a 96-well plate, this would be H12.
- **Nb Columns** is the number of vertical columns of wells when viewing the plate in its normal orientation. In the case of a 96-well plate, this would be 12.
- **Nb Rows** is the number of horizontal rows of wells when viewing the plate in its normal orientation. In the case of a 96-well plate, this would be 8.
- **Well Diameter** is the diameter of any well.
- **Height** is the distance from the bottom mounting surface of the plate to the top face of the plate.

Microplate Measurement

When creating or updating plate dimensions, it is essential that precise measurements be taken. Use calipers with precision to 0.01 microns to gather the following measurements. *A standard ruler is not precise enough.*

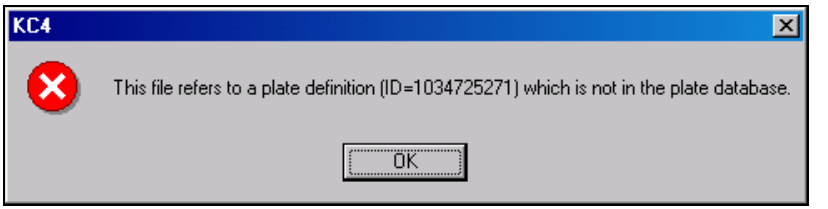
Important! Dimension values are used by the software to calculate reading positions. Any inaccuracies in these dimensions could significantly affect your results.

The following diagram demonstrates the proper method for taking measurements for a standard 96-well plate.



Exporting/Importing Plate Formats

If the following message appears when opening a protocol created with a 3.0x/2.x version of KC4, the protocol references a **custom plate format** not currently found in KC4:



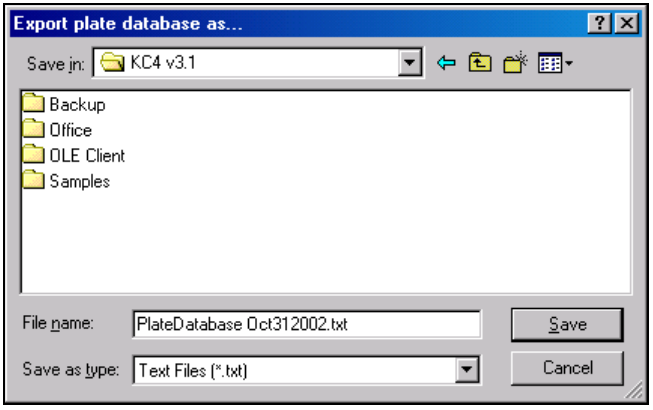
If you wish to work with 3.0x/2.x protocols that reference custom plate formats, you have two options:

#1 Export the current plate format information into a text file, manually add the custom plate format(s) to this file, and then re-import the file back into the shared database. This will both preserve your current plate format information and add the custom plate formats.

#2 Import the plate database (KC4_32.pld) from the earlier KC4 version. **Warning!** This will overwrite the current plate format information in the shared database.

To manually update KC4 with the custom plate formats from the earlier KC4 version (option #1):

1. Locate the **KC4_32.pld** file containing the desired custom plate formats (this file can be found in the root KC4 3.0x/2.x folder). Open this file using a text editor such as Notepad. Keep this file open in the background.
2. Launch KC4 v3.4 / KC4 *Signature*.
3. Select System|Plate Formats and click **Export.** The **Export plate database as** dialog will appear. Tip: If the Plate Formats option is not available in the System Menu, this means that the currently selected reader does not support customizable plate formats. Select System|Readers and choose a more appropriate reader.



4. Using the file tree, choose a target location. Enter an identifying file name and click **Save.**
5. Open this file using a text editor such as Notepad.
6. Switch to the open KC4_32.pld file. Locate the custom plate format; it will resemble the following:

   1034789489=Custom Format 1;126570,84830;25150,22860,102310,61740;2,3;34990;Bio-Tek;12345

7. Highlight the entire line, click the right mouse button, and select **Copy.**
8. Switch to the open <plate database>.txt file. Scroll to the end and place the cursor on the first available blank line. Click the right mouse button and select **Paste**. The custom plate format should appear.
9. Save the <plate database>.txt file and close it. Return to KC4.
10. Within the System|Plate Formats dialog, click **Import.** Locate the modified <plate database>.txt file and click **Open.** The Plate Formats list will update to show the contents of this file.

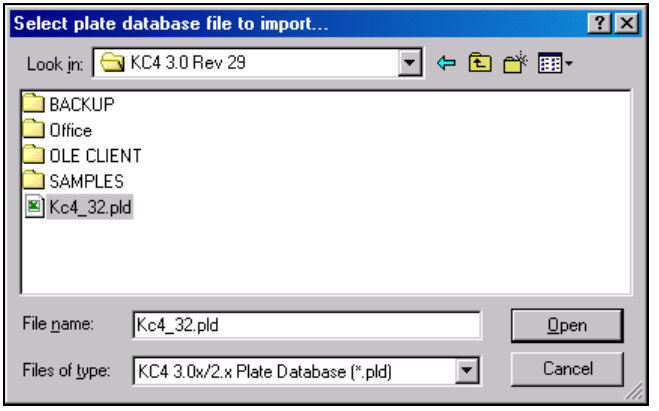
    Important: KC4 does not verify that the imported file has a valid structure or contains valid values. It is the user's responsibility to verify the structure and content of the plate formats after they have been imported.

11. Click **OK** to return to the main view.

    Note: KC4 version 3.02 introduced a new **Plate Height** dimension for plate formats. This is the distance from the bottom mounting surface of the plate to the top face of the plate. If you are importing a plate database from an earlier KC4 version, be sure to measure the plate height and add it via the **Modify** button in the Plate Formats dialog.

To import the plate database from the earlier KC4 version (option #2):

1. Launch KC4 v3.4 / KC4 *Signature*.
2. Select System|Plate Formats and click **Import.** The **Select plate database file to import** dialog will appear. Tip: If the Plate Formats option is not available in the System Menu, this means that the currently selected reader does not support customizable plate formats. Select System|Readers and choose a more appropriate reader.



3. Set **Files of Type** to 'KC4 3.0x/2.x Plate Database (*.pld)'.
4. Use the file tree to locate the KC4_32.pld file from the previous KC4 version, and then click **Open.** The Plate Formats list will update to show the contents of this file.

   Important: KC4 does not verify that the imported file has a valid structure or contains valid values. It is the user's responsibility to verify the structure and content of the plate formats after they have been imported.
5. Click **OK** to return to the main view.

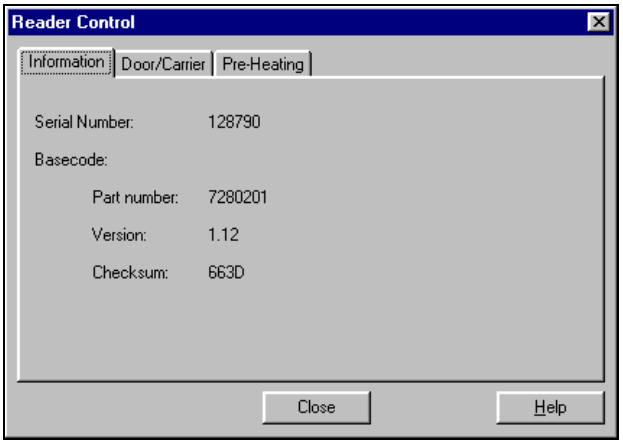
   Note: KC4 version 3.02 introduced a new **Plate Height** dimension for plate formats. This is the distance from the bottom mounting surface of the plate to the top face of the plate. If you are importing a plate database from an earlier KC4 version, be sure to measure the plate height and add it via the **Modify** button in the Plate Formats dialog.

Reader Control

If supported by the current reader, **Reader Control** is available under the System menu. This feature can be used to view information about the attached reader, control the reader door or carrier, and to control incubation.

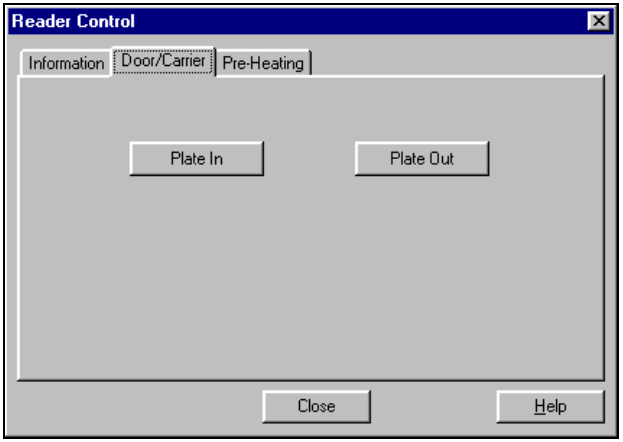
Information

Before contacting Bio-Tek for technical assistance, use the **Information** dialog to retrieve the serial number, part number, software version, and basecode checksum from the attached reader.



Door/Carrier

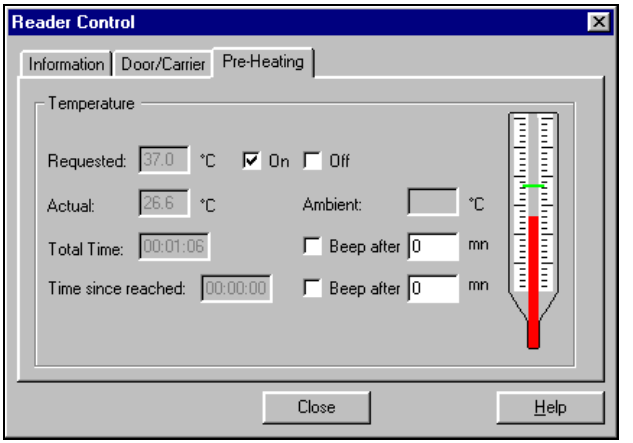
The Door/Carrier functions are reader-dependent. The attached reader must have robotic capability (such as the ELx808™ or FL600™).



- Select **Plate In** to move the plate carrier into the plate reading chamber and close the door (if applicable).
- Select **Plate Out** to move the plate carrier out of the plate reading chamber and open the door (if applicable).

Pre-Heating

Use the **Pre-Heating** dialog to set the reading chamber temperature, turn the incubation unit on or off, or to set a timer to beep after a specified duration or when the requested temperature has been reached.



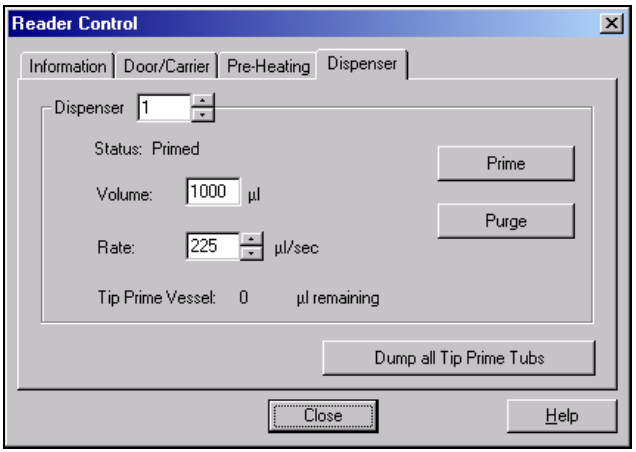
- To set the reading chamber temperature, enter the temperature in the **Requested** field and then check **On** to begin pre-heating. **On** enables the incubation unit; the temperature will rise or fall to the Requested temperature, as appropriate. **Off** disables the incubation unit; the temperature will fall to ambient.
- The **Actual** field reports the current temperature (Celsius) of the incubation unit.
- For the FL600™, the **Ambient** field reports the current temperature of the outside environment, via a sensor located within the reader (refer to the FL600 user manual for sensor location information).
- The **Total Time** field reports the total time elapsed since the **On** box was checked. Associated with the **Total Time** field, the **Beep after** checkbox and field can be enabled to request that KC4 "beep" continuously after a specified duration. The duration begins when the incubation unit is turned on.
- The **Time since reached** field reports the total time elapsed since the incubation temperature has reached the **Requested** temperature. Associated with the **Time since reached** field, the **Beep after** checkbox and field can be enabled to request that KC4 "beep" continuously after a specified duration. The duration begins when the incubation temperature reaches the **Requested** temperature.
- The **REACHED** indication appears when the incubation temperature has reached the Requested temperature.

Note: As the temperature of the incubation unit approaches the **Requested** temperature, it may take a few minutes to settle within an acceptable tolerance (+/- 0.5° C). During this settling period, the **REACHED** indication may appear for a few seconds, disappear, then reappear moments later. The **Time since reached** field will automatically reset to 00:00:00 each time the REACHED indication appears. If the **Beep after** checkbox associated with **Time since reached** field is enabled and set to, for example, 10 minutes, KC4 will "beep" 10 minutes after the incubation temperature has consistently settled within +/- 0.5° C of the **Requested** temperature.

Dispenser

Synergy™ HT with injectors

Use the **Dispenser** dialog to perform dispenser-specific functions such as prime and purge.



Priming the Dispenser

Before running a Read & Dispense protocol, the syringe(s) and tubing must be primed with the fluid to be used.

To prime the dispenser:

- Fill the supply bottle with a sufficient volume of the fluid to be used for the prime.
- **Important!** Place the priming plate on the carrier (Bio-Tek PN 7142002).
- In KC4, select **System|Reader Control** and click the **Dispenser** tab.
- Select the **Dispenser** (1 or 2) associated with the supply bottle.
- Enter the volume to be used for the prime, from 5 to 5000 µl.
- Select a prime **Rate** in µl/second.
- Click **Prime** to start the process.
- When the process is complete, carefully remove the priming plate from the carrier and dispose of the fluid.

Purging the Dispenser

KC4 provides a special utility to purge fluid from the dispense tubing and syringes by pumping the fluid in reverse, back into the supply bottle.

To purge the dispenser:

- In KC4, select **System|Reader Control** and click the **Dispenser** tab.
- Select the **Dispenser** (1 or 2) associated with the supply bottle.
- Enter the desired purge **Volume** from 5 to 5000 µl.
- Select a prime **Rate** in µl/second.
- Click **Purge** to start the process.

Emptying the Tip Prime Trough

Read & Dispense protocols can specify a tip prime to compensate for any fluid loss at the dispense tip due to evaporation since the last dispense. Tip priming is performed in a small, removable priming trough located in the left rear of the carrier. The prime trough holds up to 1 ml of liquid and must be periodically emptied and cleaned by the user. The trough must be emptied periodically, and KC4 must be "told" that the trough has been emptied.

To empty the tip prime trough:

- Carefully remove the trough from the carrier and empty it.
- Clean the trough as required by your site and then put it back on the carrier. Refer to the Synergy HT Operator's Manual for decontamination instructions.
- In KC4, select **System|Reader Control** and click the **Dispenser** tab. The Tip Prime Vessel value shows KC4's estimate of how much fluid is in the trough.
- Click **Dump all Tip Prime Troughs.** The Tip Prime Vessel value will reset to 0.

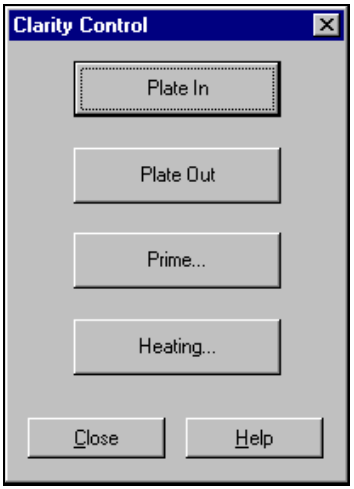
Note: If the tip priming trough overflows, refer to the Synergy HT Operator's Manual for decontamination instructions.

Note: When running a Read & Dispense protocol, KC4 may prompt you to empty the tip prime trough. In this case, KC4 will automatically open the System|Reader Control, Dispenser dialog.

Clarity Control

Clarity™ Microplate Luminometer

Use the **Clarity Control** dialog to control the microplate holder, prime the reagent lines and injectors, and control plate heating.



Microplate Holder Control

The Clarity's microplate holder can be opened and closed only through software control.

- To extend the microplate holder, click **Plate Out.**
- To retract the microplate holder into the reading and heating chamber, click **Plate In.**

Note: Do not forcibly push in or pull out the microplate holder! If for any reason you cannot control the holder using the software, use the Allan key supplied with the instrument to extend or retract the holder. Refer to the *Clarity™ Operator's Manual* for more information.

Prime

The reagent lines and injectors should be primed with the dispensing fluid before running the protocol. Click the **Prime** button to open the **Priming Parameters** dialog.

Refer to the *Clarity Operator's Manual* for complete priming instructions.

To prime the injectors:

1. Fill the reagent bottle(s) with the fluid to be dispensed.
2. Access the **Priming Parameters** dialog. All installed injectors are displayed. Injectors marked by an asterisk (*) have yet to be initialized. Initialization takes place automatically when the injector is used.
3. Click on the injector's check box. The drop-down list boxes **Direction, Volume [µl]** and **Strokes** are enabled.

4. In the Direction list box select **To Mpl.** In the **Volume [µl]** box specify the injection volume, and in the Strokes box specify the number of cycles. The total liquid volume is displayed in the **Total Volume** column.
5. Click **<Prime>** to prime the injector(s).

Heating

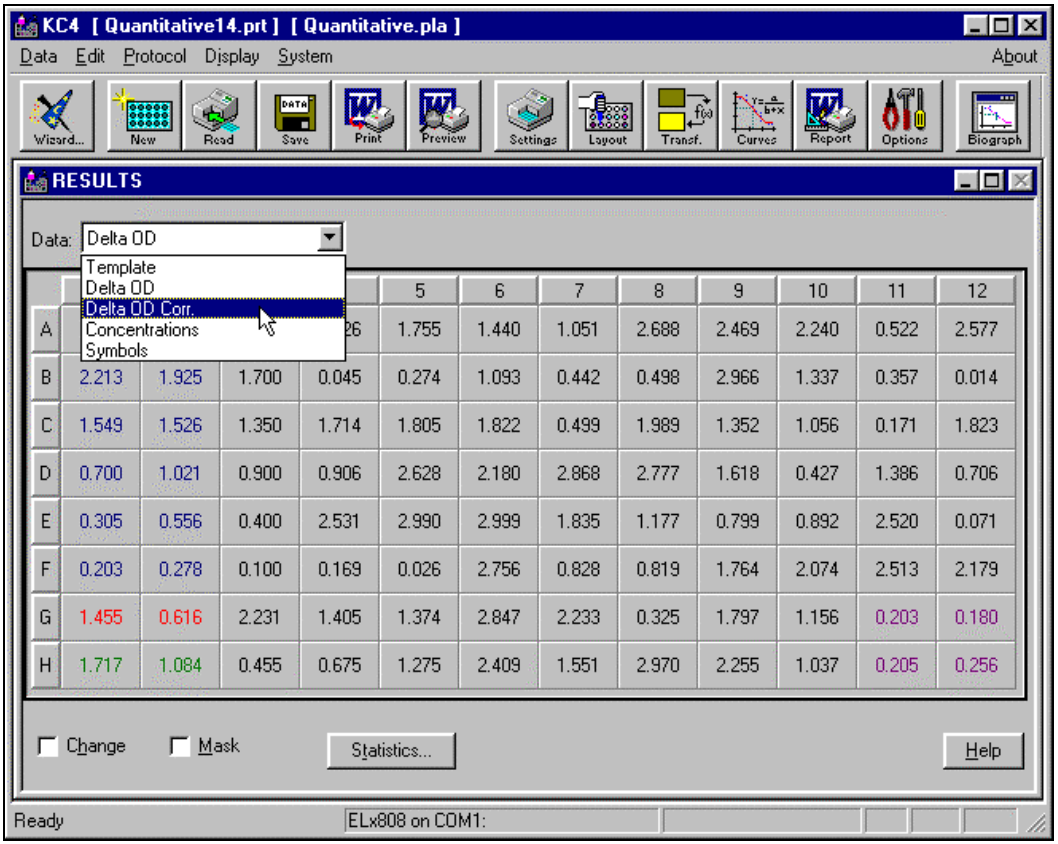
The Clarity's optional incubator is controlled via software. Click the **Heating** button to access the **Temperature Control** dialog.

Refer to the *Clarity Operator's Manual* for complete heating instructions.

Results

Overview

When plate reading is complete, or after data has been entered manually or read from a file, KC4 displays the **Results** as demonstrated below:



	1	2	3	4	5	6	7	8	9	10	11	12
A				26	1.755	1.440	1.051	2.688	2.469	2.240	0.522	2.577
B	2.213	1.925	1.700	0.045	0.274	1.093	0.442	0.498	2.966	1.337	0.357	0.014
C	1.549	1.526	1.350	1.714	1.805	1.822	0.499	1.989	1.352	1.056	0.171	1.823
D	0.700	1.021	0.900	0.906	2.628	2.180	2.868	2.777	1.618	0.427	1.386	0.706
E	0.305	0.556	0.400	2.531	2.990	2.999	1.835	1.177	0.799	0.892	2.520	0.071
F	0.203	0.278	0.100	0.169	0.026	2.756	0.828	0.819	1.764	2.074	2.513	2.179
G	1.455	0.616	2.231	1.405	1.374	2.847	2.233	0.325	1.797	1.156	0.203	0.180
H	1.717	1.084	0.455	0.675	1.275	2.409	1.551	2.970	2.255	1.037	0.205	0.256

- Click the **Data** drop-down arrow to view a different data set.
- Click **Statistics** to view statistical information for the identifiers assigned to this plate.
- Check **Change** to modify one or more raw data values, then click OK to recalculate and revalidate results. **Note:** The System Administrator can protect the use of the Change function.

- Check **Mask** to mask one or more wells to temporarily exclude them from calculations. Click on the desired well(s) then click **OK** to recalculate and revalidate results. To **unmask** a well, click Mask, click on the well, then click OK. Results will automatically recalculate.

  Important! Masking wells may significantly change or even invalidate the results. The System Administrator can protect the use of this function.

- To **copy** data to another application, highlight the desired wells then select **Copy** from the **Edit** menu. Switch to the target application and select a **Paste** option.

- For all instruments except the FL600, a well will contain **'?????'** if any of the following conditions occurs:

  The plate layout specifies the contents of a well that is not read.

  The absorbance value for the well cannot be calculated (absorbance reads only).

  The RFU/RLU value exceeds 99998 (fluorescence and luminescence reads only). **Tip:** If many or most of the wells contain **'?????'** the sensitivity setting in the protocol may be too high.

  Note: Wells containing **?????** are excluded from transformation equations and statistical calculations.

- For the FL600™, a well will contain **'?????'** if the plate layout specifies the contents of a well that is not read, or if the absorbance value for the well cannot be calculated (absorbance reads only).

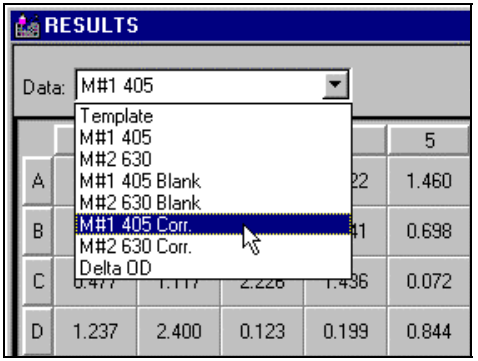
  Note: Wells containing **?????** are excluded from transformation equations and statistical calculations.

- For the FL600™ a well will contain **'OVRFLW'** if the absorbance value for a well can be calculated, but it is greater than 4.0 O.D. (absorbance reads only), or if the RFU/RLU value exceeds 99999 (fluorescence and luminescence reads only). **Tip:** If many or most of the wells contain **'OVRFLW',** the sensitivity setting in the protocol may be too high.

  Note: Wells containing **OVRFLW** are excluded from transformation equations and statistical calculations.

Data Sets

The Results data sets presented for viewing depend on the current protocol parameters. Click the **Data** menu drop-down arrow to choose from a list of available data sets.

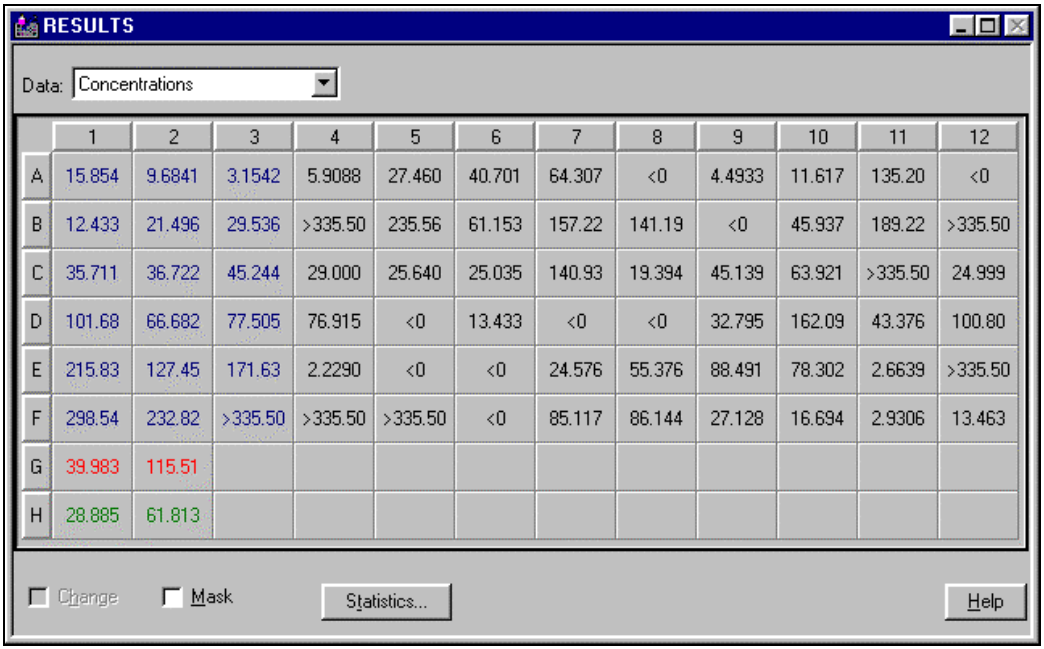


The following describes just a subset of the many different Results data sets:

- **Template** displays the identifier and any concentration or dilution value assigned to each well under the Protocol|Plate Layout dialog.
- **M 977 (SP)** and **M 900 (SP)** contain the measurement values of the Sample Plate at 977 nm and 900 nm, respectively (necessary for **pathlength correction** calculation).
- Measurement numbers **(M#1, M#2, … M#6)** correspond with the wavelength selection order in the Reading parameters dialog.
- A data set resembling **M#1 405** represents the raw measurement values for the Sample Plate at this wavelength.
- A data set resembling **M#1 405 Blank** represents the measurement values for the Blank Plate at this wavelength.
- A data set resembling **M#1 405 Corr.** represents the corrected values after Blank Plate Subtraction, Blank Wells Subtraction, and/or Pathlength Correction.
- The **Delta OD** data set represents the difference in measurement values between two wavelengths.
- The **Concentrations** data set represents the calculated concentration values. (Launch Biograph to see the curve.)
- The **Conc./Dil.** data set contains values calculated by multiplying the interpolated concentrations by any dilution factor imposed on the concentration.
- The **Symbols** data set contains the cut-off symbols assigned in accordance with the cut-off criteria defined in the protocol.
- The **Kinetic Curves** and **Scanning Curves** data sets contain the graphical representation of the measurement values in each well.
- The results of any **single-** or **multi-plate transformation** can be identified by the name defined within the Protocol| Single Plate Transformation dialog or the Protocol|Multi Plate Transformation dialog. For example, if the name of a single plate transformation is '% toxicity' select the '% toxicity' data set to view the results.

Concentrations

Concentrations are reported if standards are defined in the Plate Layout and the curve generated successfully.



	1	2	3	4	5	6	7	8	9	10	11	12
A	15.854	9.6841	3.1542	5.9088	27.460	40.701	64.307	<0	4.4933	11.617	135.20	<0
B	12.433	21.496	29.536	>335.50	235.56	61.153	157.22	141.19	<0	45.937	189.22	>335.50
C	35.711	36.722	45.244	29.000	25.640	25.035	140.93	19.394	45.139	63.921	>335.50	24.999
D	101.68	66.682	77.505	76.915	<0	13.433	<0	<0	32.795	162.09	43.376	100.80
E	215.83	127.45	171.63	2.2290	<0	<0	24.576	55.376	88.491	78.302	2.6639	>335.50
F	298.54	232.82	>335.50	>335.50	>335.50	<0	85.117	86.144	27.128	16.694	2.9306	13.463
G	39.983	115.51										
H	28.885	61.813										

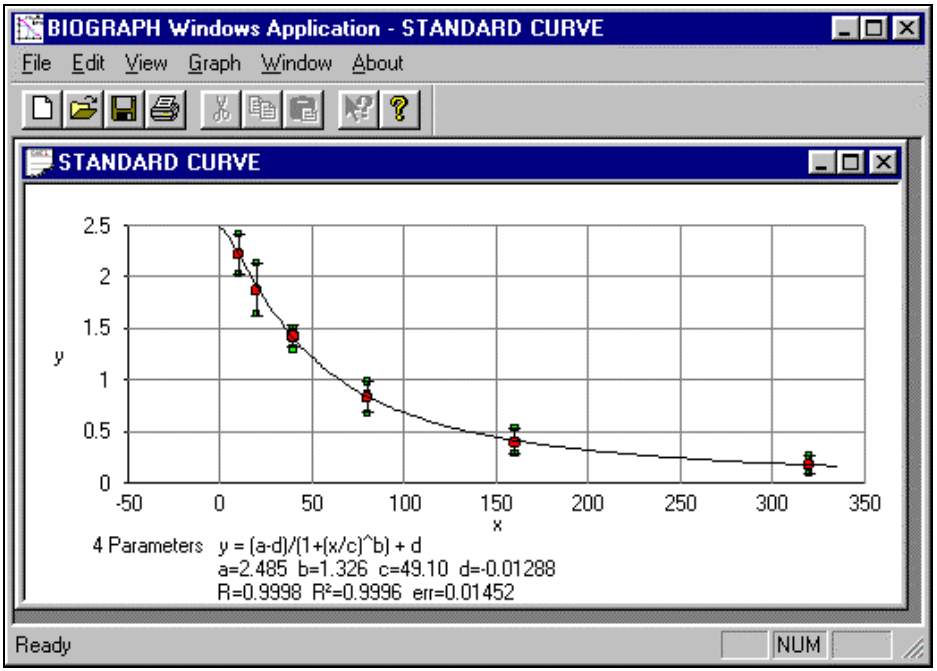
- Values outside the range of the standard curve are reported as **'<lowest conc.'** or as **'>highest conc.'** where the 'lowest conc.' and 'highest conc.' represent the minimum and maximum concentrations plotted on the standard curve, respectively. **Note:** These two values may be different than the min/max standard concentrations if an extrapolation factor is applied.

  See *System Menu, Setup, Format* for information on using 'out of range' values in statistical calculations.

- To calculate values outside the normal limits of the curve, select **Protocol|Curves|Axes** to increase the **Extrapolation Factor.**

- If a concentration could not be calculated for a well, the **?????** symbol is reported. One reason this may occur is if the value exceeds the limits of the standard curve.

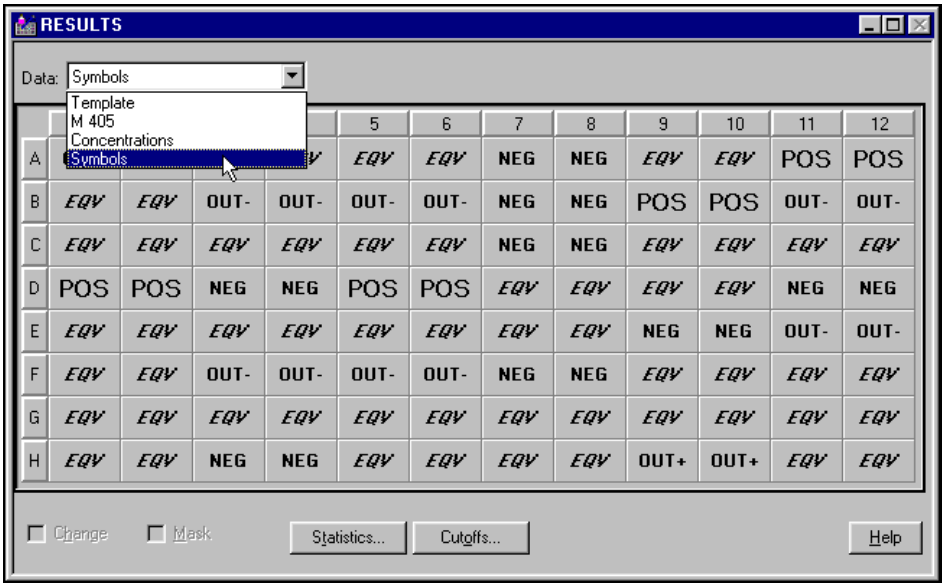
- Select **Display|Curve(s)** or click the **Biograph** toolbar button to view curves:



Symbols

If **Cutoffs** are defined in the protocol, the specified set of data is evaluated against the cutoff criteria and each well is assigned a user-defined **Symbol.**

To view the assigned symbols, select the Symbols data set:


RESULTS
Data: Symbols
Template
M 405
Concentrations
Symbols
5 6 7 8 9 10 11 12
A EQV EQV NEG NEG EQV EQV POS POS
B EQV EQV OUT- OUT- OUT- OUT- NEG NEG POS POS OUT- OUT-
C EQV EQV EQV EQV EQV EQV NEG NEG EQV EQV EQV EQV
D POS POS NEG NEG POS POS EQV EQV EQV EQV NEG NEG
E EQV EQV EQV EQV EQV EQV EQV EQV NEG NEG OUT- OUT-
F EQV EQV OUT- OUT- OUT- OUT- NEG NEG EQV EQV EQV EQV
G EQV EQV EQV EQV EQV EQV EQV EQV EQV EQV EQV EQV
H EQV EQV NEG NEG EQV EQV EQV EQV OUT+ OUT+ EQV EQV
Change
Mask
Statistics...
Cutoffs...
Help

- If the cutoffs are in increasing order, **symbol assignment** is determined as follows:

  Each well is evaluated against the first cutoff. If the value of the well is **less than** the criteria, then the first symbol is assigned to the well. If the value of the well is **greater than or equal to** the first cutoff, then it is evaluated against the next cutoff. If the value of the well is less than the next criteria, then the symbol immediately preceding the cutoff criteria is assigned to the well.

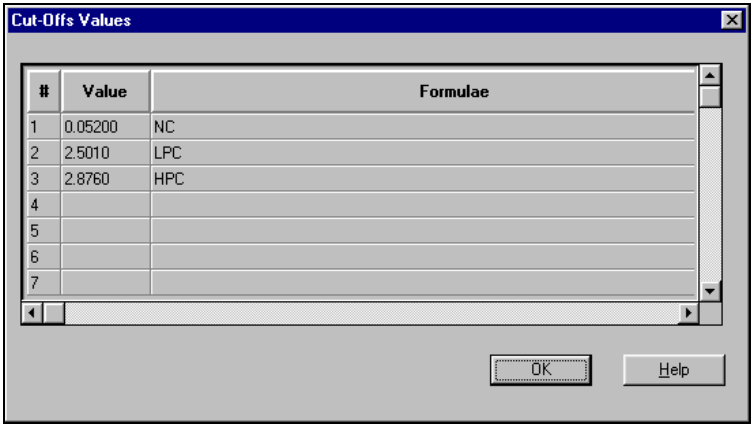
  This process continues until the last cutoff criteria is encountered. If the value of the well is less than the last criteria, then the symbol immediately preceding the last cutoff criteria is assigned to the well. If the value of the well is **greater than or equal to** the last criteria, then the symbol immediately following the last cutoff criteria is assigned to the well.

- If the cutoffs are not in increasing order, the message "Cutoffs are not in increasing order. Symbols can not be determined" appears, and **?????** is assigned to every well in the Symbols data set.

- **?????** may also appear in one or more wells if a cutoff formula references a well ID that is not defined in the Plate Layout, a well that is masked, or a well that is out-of-range or has been failed by the attached microplate reader.

- If a well contains **OUT-** or **OUT+** cutoffs are based on concentrations and the well fell below or above the lowest or greatest X value of the calculated standard curve.

- Click the **Cutoffs** button to see the cutoff criteria and the calculated cutoff value(s).



Cutoffs Values

The contents of the **Formulae** in this dialog correspond with the **Cutoffs** defined within the protocol.

The calculated value of each cutoff is displayed in the **Value** column.

If a Value is **?????** the cutoff could not be calculated. Possible causes include:

- A cutoff formula references a well ID that is not defined in the Plate Layout.
- A cutoff formula references a well that is masked.
- A cutoff formula references a well that is out-of-range or has been failed by the attached microplate reader.

Pathlengths

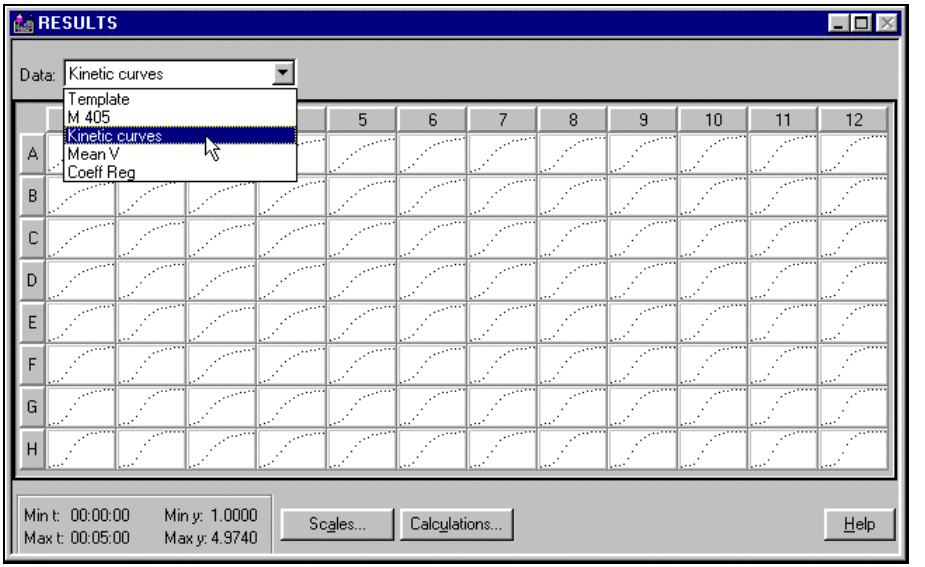
Pathlength Correction can be enabled to normalize absorbances to 1 cm. Its use is controlled within the **Protocol|Reading parameters** dialog.

If Pathlength Correction is enabled, KC4 first calculates the **pathlength** for each well. The absorbance in each well is then divided by the pathlength, resulting in the **corrected absorbance.** When calculations are complete, KC4 automatically reports the corrected values, available by selecting **M 405 Corr., M 630 Corr.,** etc. from the Data drop down list.

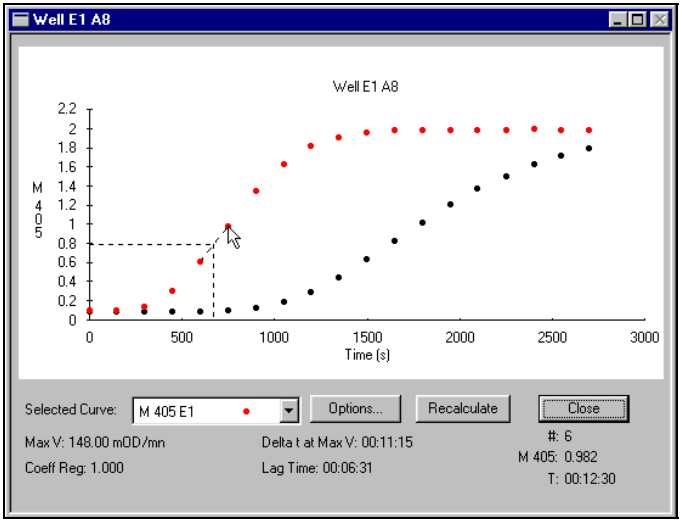
You can easily create a **Multi Plate Transformation** to report the pathlength of each well. You can give the transformation a descriptive name such as "Pathlengths" and the resulting data set will be available from the Data drop down list. To learn how to do this, access KC4's help system contents and select Results > Data Sets > Pathlengths. Click on "Click here for more information on reporting the pathlength for each well."

Kinetic Data

KC4 provides several options for viewing kinetic data. The default view is **Kinetic Curves.**



- Each well displays a graph of the measurement values taken over time.
- Hold down the **Ctrl** key and click on up to **8** wells to **overlay** the kinetic curves in one display.
- Click the **Scales** button to adjust the data points displayed for each well. The current scaling parameters are shown in the lower left corner of the Kinetic Curves display.
- Click the **Calculations** button to alter the kinetic calculation zone for all wells.
- Click on a well to **enlarge** the view of its kinetic curve:

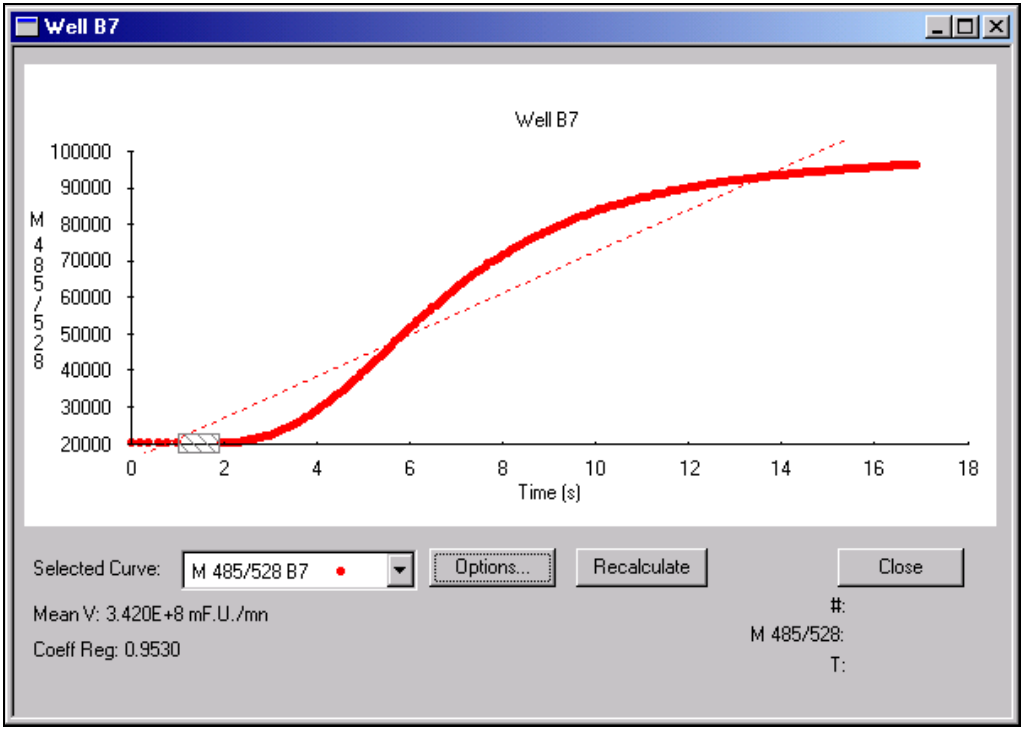


- This dialog can display up to 8 overlaid kinetic curves.
- **Kinetic calculations** such as Max V, Coeff Reg, Delta t at Max V, and Lag Time associated with the currently selected plot are displayed. Click on the down arrow associated with **Selected Curve** to choose a different curve.
- Moving the mouse cursor over a kinetic data point in the currently selected curve will display coordinates for that point. Data displayed can include **Read #, OD, and Reading Time.**
- Click on an individual data point to temporarily **exclude** it from further calculations, then click the **Recalculate** button to exclude the point from kinetic calculations. **Important!** Modifying data may adversely affect the final results.
- To **restore** an excluded data point, click on the point and then click **Recalculate.**
- Click the **Options** button to adjust the **Kinetic Analysis** parameters for the curves shown in this dialog. Refer to **Kinetic Analysis** under "Protocol Reading Parameters" in the *Protocol Menu* section of this guide for more information.

Dispense During Kinetic Read

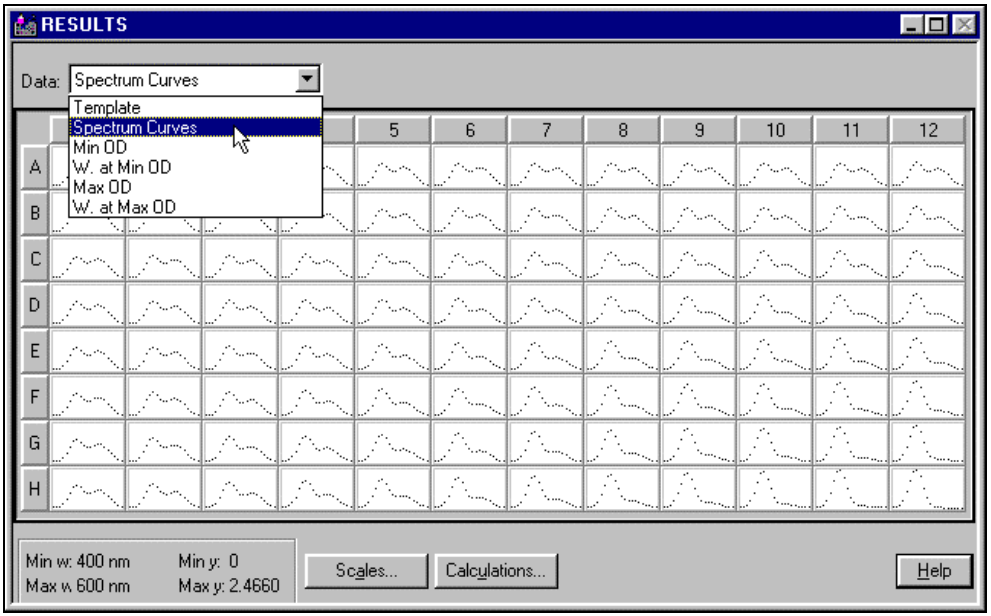
The Synergy™ HT with injectors model provides the ability to dispense fluid into microwells as part of a fluorescence or luminescence Read & Dispense protocol.

When the protocol specifies a dispense operation during a kinetic read, the Kinetic Curves display will represent the dispense operation in a box with cross hatches on the Time axis, as shown below. No readings are taken during a dispense operation.



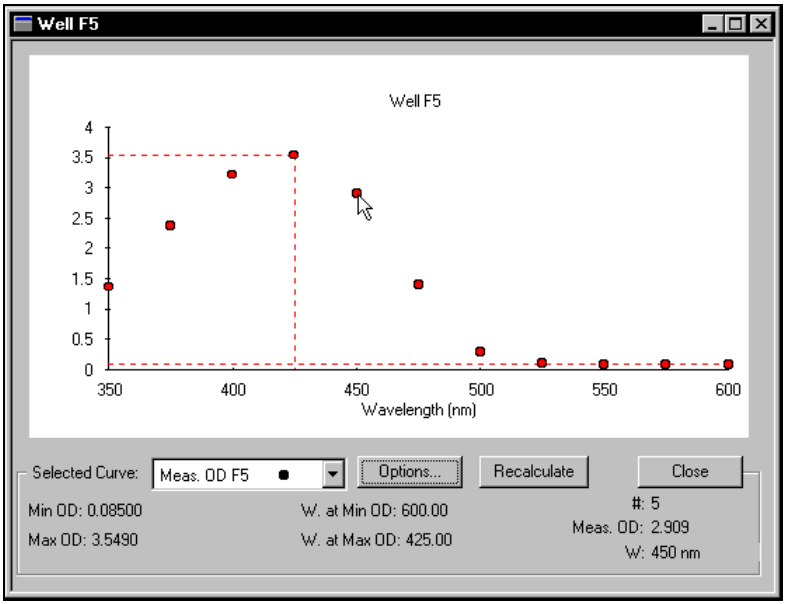
Spectral Scan Data

The default view for spectral scan data is **Spectrum Curves:**



- Each well displays a graph of the measurement values taken across a wavelength range.
- Hold down the **Ctrl** key and click on up to **8** wells to **overlay** the spectrum curves in one display.
- Click the **Scales** button to adjust the data points displayed for each well. The current scaling parameters are shown in the lower left corner of the Spectrum Curves display.
- Click the **Calculations** button to alter the spectrum calculation zone for all wells.

- Click on a well to **enlarge** the view of its spectrum curve.

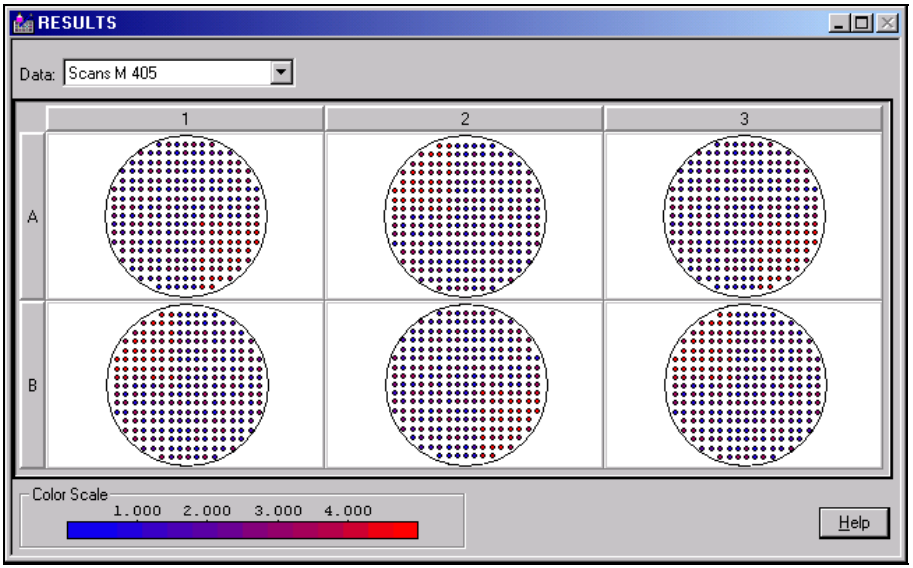


  - The spectrum curve initially contains all values measured at all wavelengths as defined in the protocol. Note: If a data point is "missing" for a particular wavelength, this most likely means that the measurement value over ranged.
  - **Spectrum calculations** such as Min OD/Max OD and W at Min OD/Max OD, associated with the currently selected plot are displayed. Click on the down arrow associated with **Selected Curve** to choose a different curve.
  - Moving the mouse cursor over a data point in the currently selected curve will display coordinates for that point. Data displayed can include **Wavelength #, OD,** and **Wavelength value** in nanometers.
  - Click on an individual data point to temporarily **exclude** it from further calculations, then click the **Recalculate** button to exclude the point from calculations.

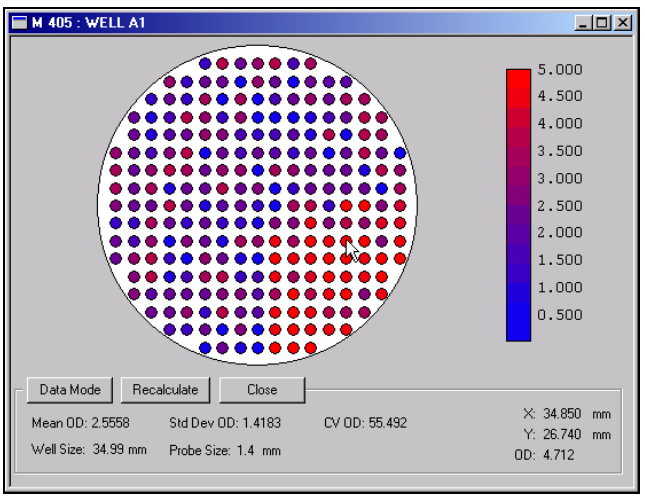
    Important! Modifying data may adversely affect the final results.
  - To **restore** an excluded data point, click on the point then click **Recalculate.**
  - Click the **Options** button to adjust the **Spectrum Analysis** parameters for the curves shown in this dialog.

Area Scan Data

The default view for area scan results contains the graphical representation of the measurements taken down and across each well:



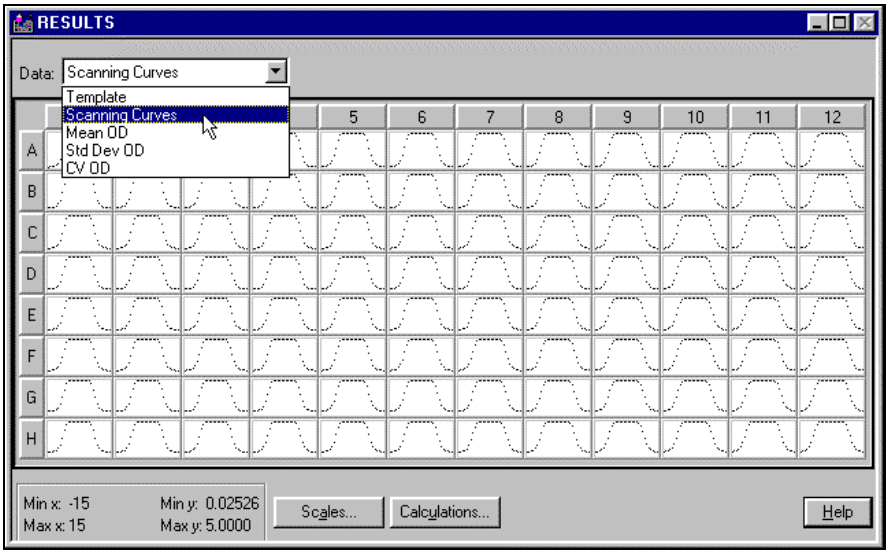
- To view a different set of data, click the Data drop-down arrow and select **Mean** OD/RFU/RLU, **Std Dev** OD/RFU/RLU, or **CV** OD/RFU/RLU.
- Click on a well to **zoom** in on it:



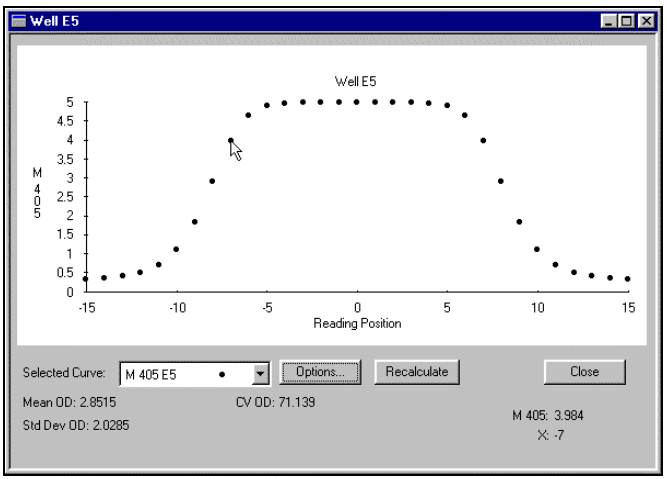
  - **Picture Mode** (the default view) is a graphical representation of all measurements taken across the well. Place the cursor over any point in the well to see its value and its X/Y location on the plate. Click **Data Mode** to view the measurement values for all points.
  - To exclude/restore a point from the scanning calculations, click on the desired point and then click **Recalculate.**

Linear Scan Data

The default view for linear scan results contains the graphical representation of the measurements taken across each well:



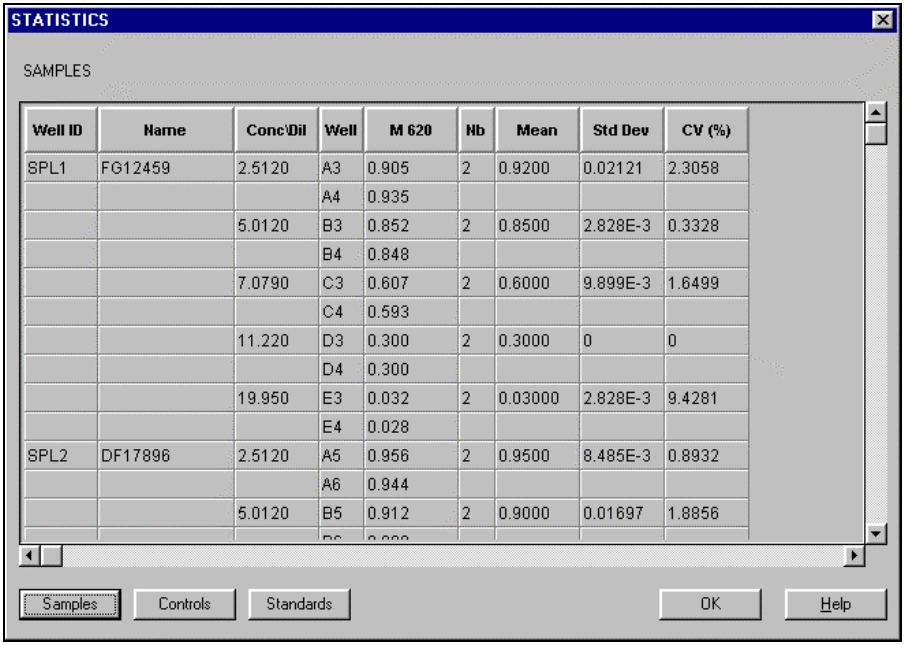
- To view a different set of data, click the Data drop-down arrow and select **Mean OD, Std Dev OD,** or **CV OD.**
- Click on a well to **zoom** in on it:



  - Place the cursor over any point in the well to see its measurement value and its X location in the well.
  - If the graph contains more than one curve, use the **Selected Curve** menu to choose a different curve and view its Mean OD, Std Dev OD, and CV OD values.
  - Click **Options** to adjust the Calculation Zone, then click **Recalculate** to update the calculations.

Statistics

From within any Results window, click the **Statistics** button to view the statistical data associated with the results.



Well ID	Name	Conc\Dil	Well	M 620	Nb	Mean	Std Dev	CV (%)
SPL1	FG12459	2.5120	A3	0.905	2	0.9200	0.02121	2.3058
			A4	0.935				
		5.0120	B3	0.852	2	0.8500	2.828E-3	0.3328
			B4	0.848				
		7.0790	C3	0.607	2	0.6000	9.899E-3	1.6499
			C4	0.593				
		11.220	D3	0.300	2	0.3000	0	0
			D4	0.300				
		19.950	E3	0.032	2	0.03000	2.828E-3	9.4281
			E4	0.028				
SPL2	DF17896	2.5120	A5	0.956	2	0.9500	8.485E-3	0.8932
			A6	0.944				
		5.0120	B5	0.912	2	0.9000	0.01697	1.8856

- **Tip:** To view statistics for a different data set, go back to the main Results window, choose the desired data set and then click Statistics.
- To view statistics for a different set of identifiers, click **Samples, Controls,** or **Standards.**
- The available columns are protocol-dependent:

  Name: The name of the sample associated with the current well.

  Conc.\Dil.: Concentration or dilution of the current well.

  Well: Coordinates of the current well.

  Nb: Represents the number of replicates that were used to calculate statistics.

  Mean: Mean of the replicate group.

  Std Dev: Standard deviation of the replicate group.

  CV (%): Coefficient of variation of the replicate group, i.e. the standard deviation expressed as a percentage of the mean.

Biograph

Overview

Biograph.exe

The KC4 software package includes the **Biograph** module for curve viewing and manipulation. Biograph can be used either as a stand-alone product or in conjunction with KC4.

The Biograph functions are described in the following sections.

- To start Biograph from within KC4 and view the current curve(s), select **Display|Curves** or click the **Biograph** toolbar button.
- To launch Biograph in stand-alone mode, select **Biograph.exe** from within the KC4 folder.

Both KC4 and Biograph allow you to select the mathematical curve that best fits the standards, and this curve is used for calculating unknowns. Each curve is displayed with its formula and the calculated coefficients and correlation parameters.

File Menu

Use the **File Menu** to work with curves and graph (.gph) files.

Creating a New Curve

Select **File|New** to define a new curve by manually entering the coordinates of the standard points.

- Enter the abscissa values in the **x** column. These are typically the standard concentration values.
- Enter the ordinate values in the **y** columns. These are typically the measurement values (OD, RFU, RLU). You can enter up to 12 'y' values (replicates) per standard.

  The **M y** column calculates the mean for each row of y values. **Note:** The curve is calculated based on the mean y values, not the individual replicate values.

  The **SD y** column calculates the standard deviation for each row of y values.
- To **mask** a y value, check the Mask box then click on the desired y value. It will temporarily change to *****.
- To **unmask** a y value, keep the Mask box checked and click on the desired *****. It will change back to its original value.
- Click **OK** to display the new curve.

Closing a Curve

Select **File|Close** to close the current curve.

- If the current curve is one generated dynamically using data from KC4, clicking Close will close both the curve and the Biograph application.
- If the current curve is a stored .gph file, clicking Close will close the file but leave Biograph open.

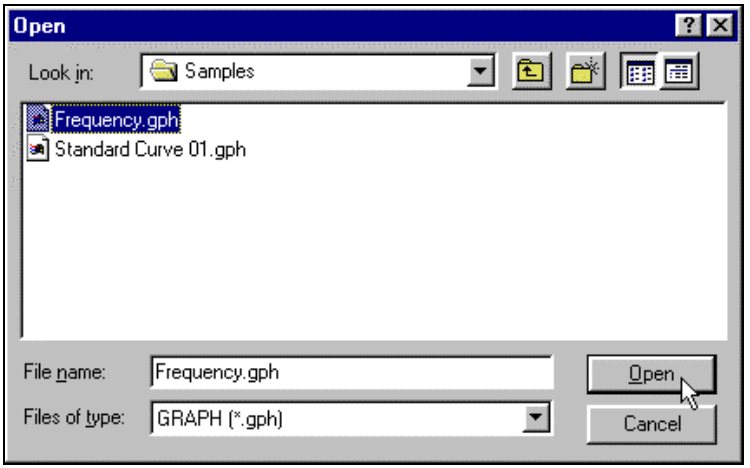
Saving a Curve (Graph File)

Select **File|Save** or **File|Save As** to save the current curve as a graph file.

- Graph files of the type ***.GPH** contain calculated curve-fitting values. A stored .GPH file can be used as the basis for calculating concentrations in a protocol that does not contain standard wells.
- **Tip:** You can use refer to this curve in KC4 as the basis for calculating concentrations in a protocol that contains no standards. To do this, select **Protocol|Curves** in KC4, then enter the name of this .GPH file in the Curve Origin, File field.

Opening a Graph File

Select **File|Open** to retrieve a stored graphic file with the extension **.GPH** and manipulate it in the same manner as newly created graphs.

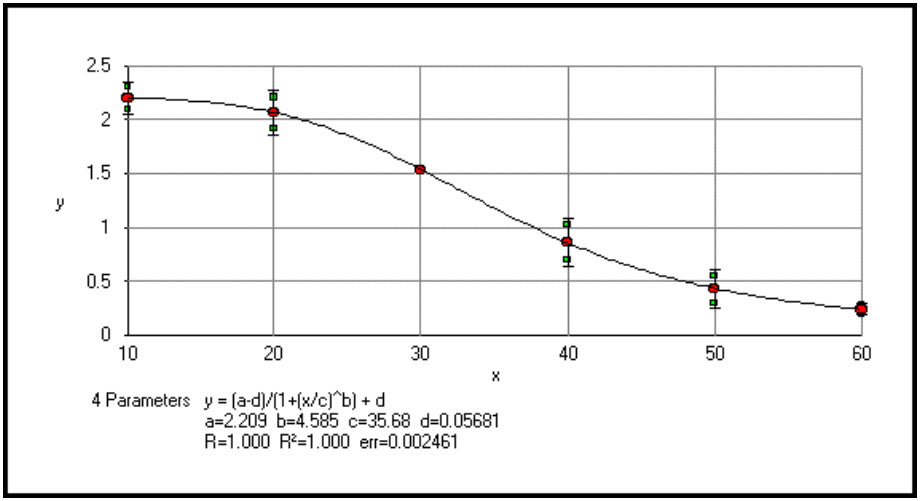


- To open a file, highlight its name then click **Open.**
- To look for a file in a different folder, click the drop-down arrow next to the **Look In** field, or click the previous folder button.

Printing Curves

Select **File|Print** to print the current curve, **File|Print Preview** to preview the printout, or **File|Print Setup** to adjust the printer settings.

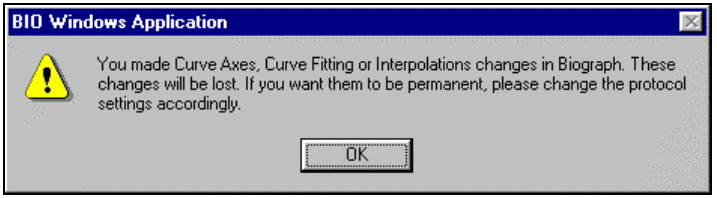
The report contains a picture of the curve, the name of the curve fit method, the equation, and the calculated coefficients.



Exiting Biograph

Select **File|Exit** to close Biograph and return to KC4 or Windows, wherever Biograph was originally launched from.

- Upon returning to KC4, all changes are lost and the following message appears:


BIO Windows Application
You made Curve Axes, Curve Fitting or Interpolations changes in Biograph. These changes will be lost. If you want them to be permanent, please change the protocol settings accordingly.
OK

View Menu

Use the **View Menu** to customize Biograph by hiding/displaying the status bar and the tool bar.

The Toolbar

By default, the **Toolbar** is displayed below the menu options:

- To hide the Toolbar, select **View|Toolbar** to remove the checkmark.
- To **redisplay** the Toolbar, select View|Toolbar to reinstate the checkmark.

The Status Bar

By default, the **Status Bar** is displayed at the bottom of the screen:

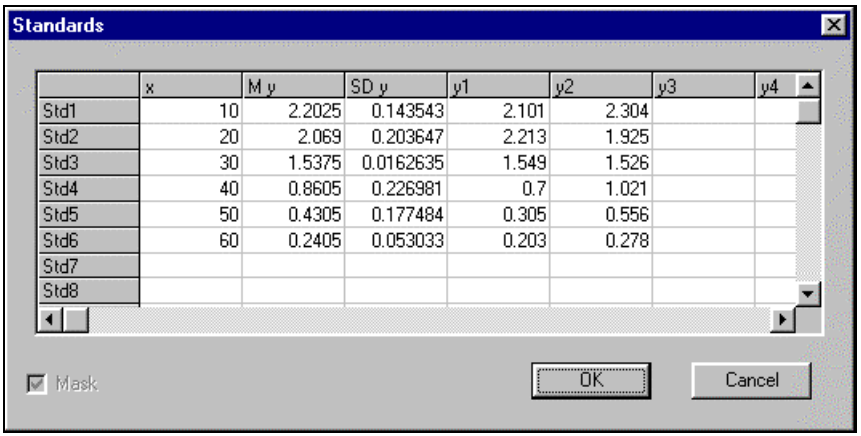
- To hide the Status Bar, select **View|Status Bar** to remove the checkmark.
- To **redisplay** the Status Bar, select View|Status Bar to reinstate the checkmark.

Graph Menu

Use the **Graph Menu** to view and/or modify certain characteristics of the current curve.

Standards

Use the **Standards** dialog to view, enter, or modify the 'x' and 'y' values of standards. **Note:** When Biograph is launched from KC4, these values cannot be modified.



	x	M y	SD y	y1	y2	y3	y4
Std1	10	2.2025	0.143543	2.101	2.304		
Std2	20	2.069	0.203647	2.213	1.925		
Std3	30	1.5375	0.0162635	1.549	1.526		
Std4	40	0.8605	0.226981	0.7	1.021		
Std5	50	0.4305	0.177484	0.305	0.556		
Std6	60	0.2405	0.053033	0.203	0.278		
Std7							
Std8							

- The abscissa values are listed in the **x** column. These are typically the standard concentration values.
- The ordinate values are listed in the **y** columns. These are typically the measurement values (OD, RFU, RLU). You can enter up to 12 'y' values (replicates) per standard.

  The **M y** column calculates the mean for each row of y values. **Note:** The curve is calculated based on the mean y values, not on the individual replicate values.

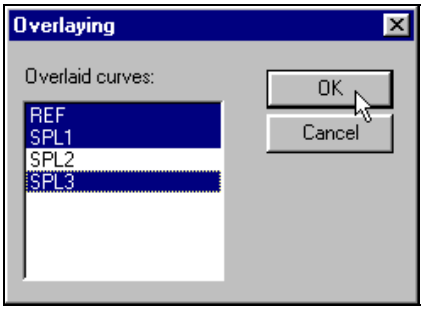
  The **SD y** column calculates the standard deviation for each row of y values.
- To **mask** a y value, check the Mask box then click on the desired y value. It will temporarily change to *****.
- To **unmask** a y value, keep the Mask box checked and click on the desired *****. It will change back to its original value.
- Click **OK** to display the curve.

Axes, Fit, Interpolations

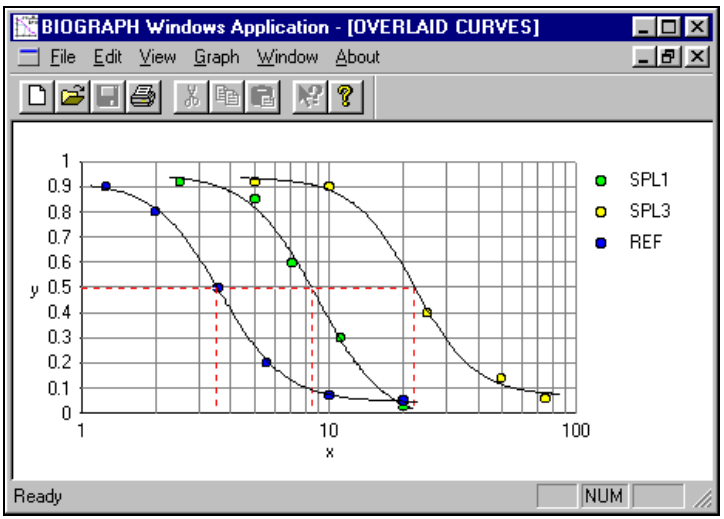
These features function similarly to functions of the same name in KC4.
See "Curves" under *Protocol Menu* for more information.

Overlaying Graphs

Biograph allows you to view multiple graphs in the same session.



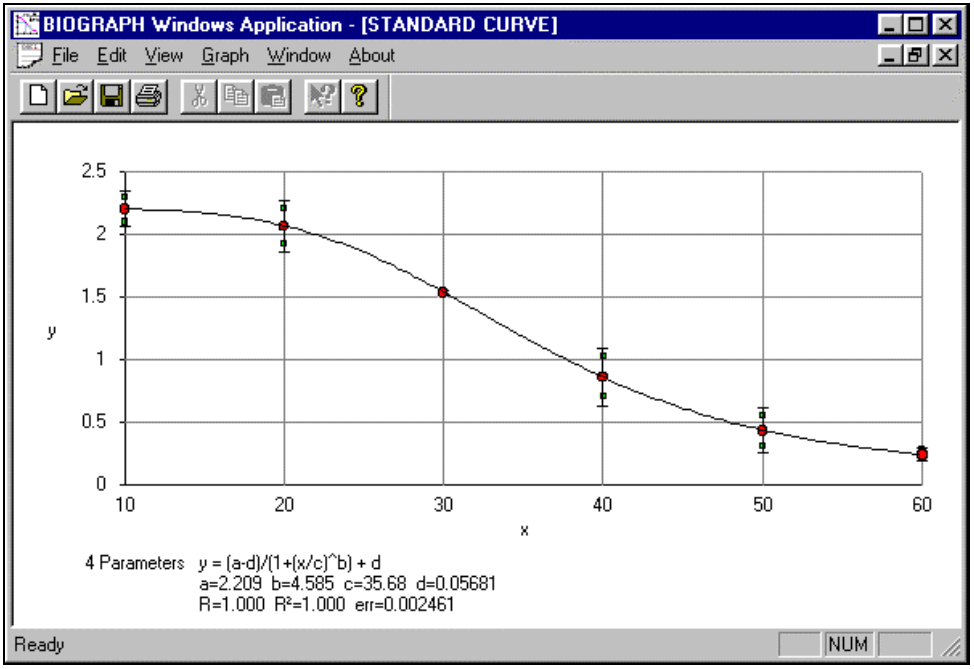
- The graphs must be **open** before they can be overlaid.
- Up to 8 graphs can be overlaid, regardless of curve fit type, but all must have the same axes (either all linear or all logarithmic). The curve overlay will display in a new window labeled **OVERLAYED CURVES.**



- The curves in this window cannot be edited. New interpolations can, however, be imposed.
- Interpolations cannot be adjusted within this window (make adjustments in the individual curve windows).
- However, any changes which you make to an individual curve or curves are reflected immediately in the "shadow curve" in the OVERLAYED CURVES window once you have calculated the change using Graph|Recalculate.

Curve Window

The **replicate** of each data point is displayed with a **green square** and the mean **replicate** value is displayed in a **red circle.**



- Click on an individual data point to temporarily **exclude** it from further calculations, then select **Graph|Recalculate** button to recalculate the curve without the point.

  Important! Modified data may adversely affect the final results.

  Changes made here are not automatically transferred over to KC4. To make permanent curve-fitting changes, return to KC4 and select **Protocol|Curves.**

- To **restore** an excluded data point, click on the point then select **Graph|Recalculate.**
- Select **File|Print** to print the graph.

PowerReports

High-Powered Reporting

Since its inception, KC4 has helped users with designing professional, high-quality reports tailored to meet their specific needs. KC4 now takes information presentation to an even higher level with **PowerReports.**

PowerReports links KC4 with Microsoft® Excel/Word 2000, providing you with a new format for creating custom report and export documents.

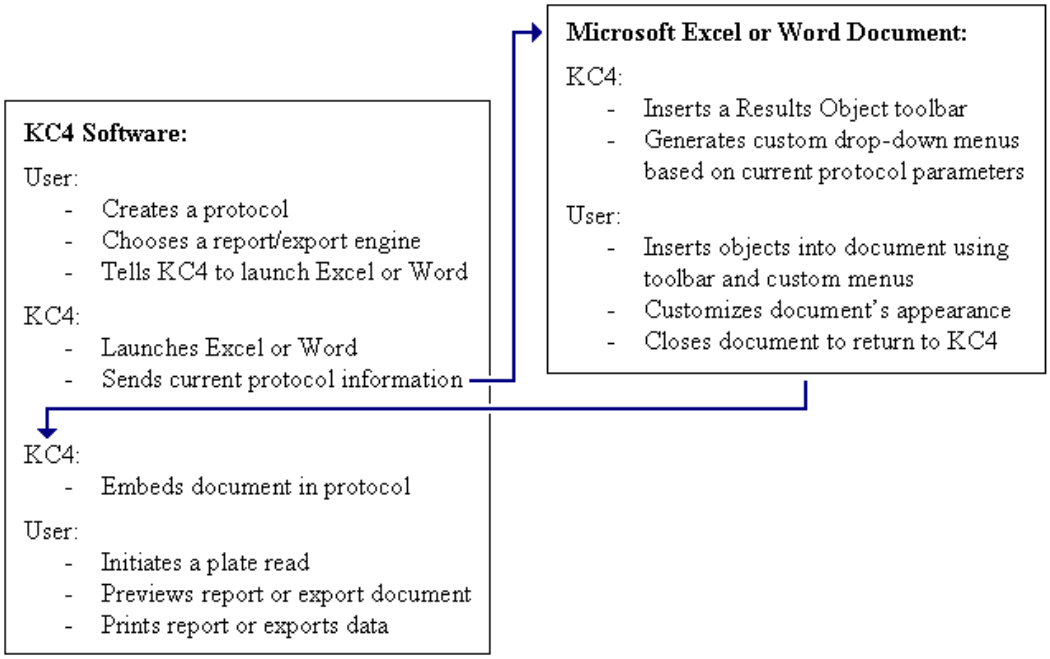
With PowerReports you can:

- Take full advantage of Excel/Word's document customization features to generate **publication-quality** reports.
- Include **pictures, drawings,** and **company logos** in reports.
- Create **custom formulas** in Excel to perform additional calculations on KC4 data.
- Take advantage of Excel's **charting capabilities** to create bar graphs, pie charts, and more.
- Perform a **direct export** of plate data to Excel without first having to create an export template.

How it Works

PowerReports uses Object Linking and Embedding (OLE) technology to interact with Microsoft Excel and Word, providing you with total control over all report formatting using **results objects** created by KC4.

KC4 sends information about the current protocol parameters to an Excel or Word **document.** *You* design the document by selecting the pieces of information to include on the report or in the export file, defining the locations for the different pieces of information, and customizing the appearance of the document. The document then becomes a part of the protocol, and is available for editing any time the protocol is open.


KC4 Software:
User:
- Creates a protocol
- Chooses a report/export engine
- Tells KC4 to launch Excel or Word
KC4:
- Launches Excel or Word
- Sends current protocol information
Microsoft Excel or Word Document:
KC4:
- Inserts a Results Object toolbar
- Generates custom drop-down menus based on current protocol parameters
User:
- Inserts objects into document using toolbar and custom menus
- Customizes document's appearance
- Closes document to return to KC4
KC4:
- Embeds document in protocol
User:
- Initiates a plate read
- Previews report or export document
- Prints report or exports data

KC4 v3.0

Highland Park, Box 998, Winooski, Vermont 05404 USA

Kit Lot: AFF52-25A **Date:** 1/31/2001

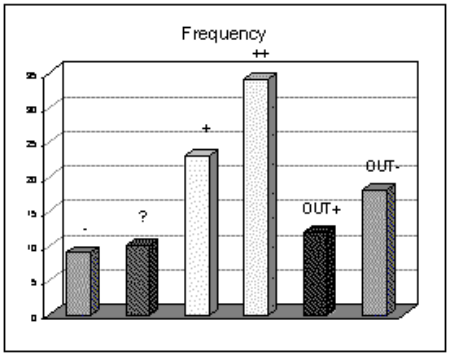
Protocol: PowerReports 1.glb
Sample Lot: 991205#402

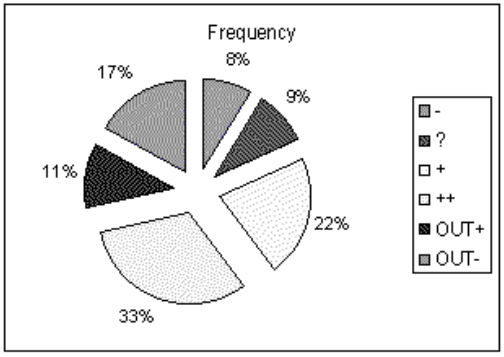
Test Validation

Assay Results

	1	2	3	4	5	6	7	8	9	10	11	12
	STD1	*STD1*	*STD1*	*SPL1*	*SPL1*	*SPL1*	*SPL2*	*SPL2*	*SPL2*	*SPL3*	*SPL3*	*SPL3*
	2.1	2.3	2.51	2.43	1.75	1.44	1.05	2.69	2.47	2.24	0.52	2.58
A				5.9	137.3	407	64.3	<0.0	44.9	11.6	676	<0.0
	?	?	-	-	+	+	++	OUT-	-	?	++	OUT-
	STD2	*STD2*	*STD2*	*SPL4*	*SPL4*	*SPL4*	*SPL5*	*SPL5*	*SPL5*	*SPL6*	*SPL6*	*SPL6*
	2.21	1.93	1.7	0.04	0.27	1.09	0.44	0.5	2.97	1.34	0.36	0.01
B				>335.5	1177.8	611.5	157.2	705.9	<0.0	45.9	946.1	>3355.0
	?	+	+	OUT+	++	++	++	++	OUT-	+	++	OUT+
	STD3	*STD3*	*STD3*	*SPL7*	*SPL7*	*SPL7*	*SPL8*	*SPL8*	*SPL8*	*SPL9*	*SPL9*	*SPL9*
	1.55	1.53	1.35	1.71	1.8	1.82	0.5	1.99	1.35	1.06	0.17	1.82
C				29	128.2	250.3	140.9	97	451.4	63.9	>1677.5	250
	+	+	+	+	+	+	++	?	+	++	OUT+	+
	STD4	*STD4*	*STD4*	*SPL10*	*SPL10*	*SPL10*	*SPL11*	*SPL11*	*SPL11*	*SPL12*	*SPL12*	*SPL12*
	0.7	1.02	0.9	0.91	2.63	2.18	2.87	2.78	1.62	0.43	1.39	0.71
D				76.9	<0.0	134.3	<0.0	<0.0	327.9	162.1	216.9	1008
	++	++	++	++	OUT-	?	OUT-	OUT-	+	++	+	++
	STD5	*STD5*	*STD5*	*SPL13*	*SPL13*	*SPL13*	*SPL14*	*SPL14*	*SPL14*	*SPL15*	*SPL15*	*SPL15*
	0.3	0.56	0.4	2.53	2.99	3	1.84	1.18	0.8	0.89	2.52	0.07
E				2.2	<0.0	<0.0	24.6	276.9	884.9	78.3	13.3	>3355.0
	++	++	++	-	OUT-	OUT-	+	++	++	++	-	OUT+

Sample Quality





System Requirements

To put the **power** in PowerReports, your computer must meet or exceed the following requirements:

- Pentium II processor (or equivalent) / 300 MHz or faster
- 64 MB RAM (128 recommended)
- Microsoft Office 2000 (9.0.3821 SR-1 or newer), Office XP, Office 2003

  To check your Office 2000 version, launch Word or Excel and select Help|About Microsoft Word (or Excel).
- Windows 98/2000/NT/XP

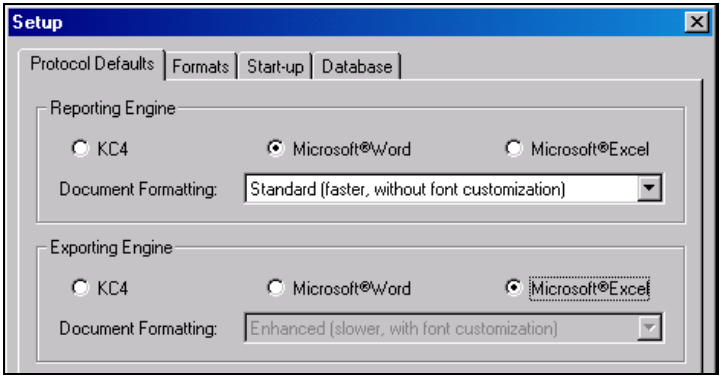
Important! If your computer does not currently meet all of these requirements, you can still print reports and export data. With KC4's standard reporting and exporting functions you can customize the contents, and to some extent the appearance, of printed reports and exported plate data. *All KC4 users have access to these standard functions.*

Preparing KC4 for PowerReporting

KC4 offers three reporting/exporting drivers, or **engines:** KC4, Microsoft Excel, and Microsoft Word. KC4 needs to know which engine(s) to you want to engage for editing, previewing, and printing reports and for editing and performing exports. The following topics explain how to select default global reporting and exporting engines and how to override them when necessary.

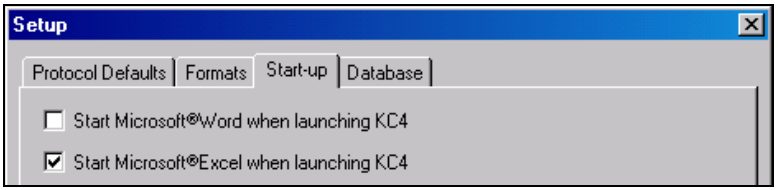
Setting Global Options

Under **System|Setup|Protocol Defaults,** KC4 allows you to select a default **Reporting Engine** and a default **Exporting Engine.** When you create a new protocol, KC4 refers to these settings to know which engine(s) to engage. These default settings *can* be overridden within a protocol (explained in the next section).



- Select the default **Reporting Engine** and **Exporting Engine** (the ones you plan to use most frequently).

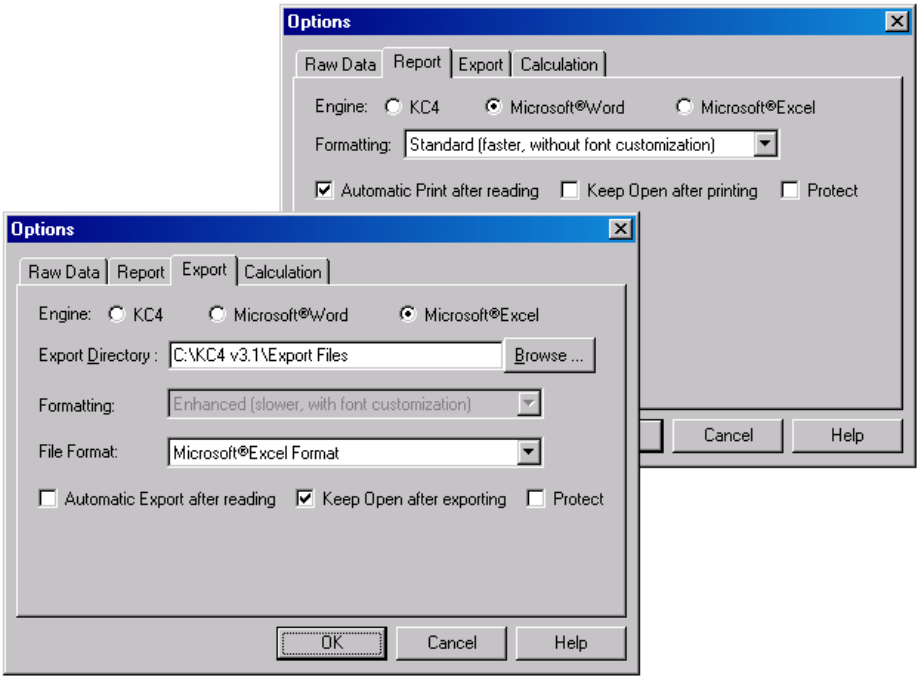
- If you have selected Microsoft Word for either or both engines, choose a Word **Document Formatting** option of Standard or Enhanced.
  - With **Standard** formatting, the print preview and print functions perform quickly but any custom Plate Layout or Cut-Offs fonts are not applied.
  - With **Enhanced** formatting, the print preview and print functions may take longer but all of your custom Plate Layout and Cut-Offs fonts will be applied.
- Click the **Start-up** tab to customize additional options:



  - (Optional) Check **Start when launching KC4** if you want Word and/or Excel to open automatically when KC4 is launched. With the program open in the background, document opening and formatting will perform more quickly.

Setting Protocol-Specific Options

If you have opened a protocol and want to change the reporting or exporting engine but do not want to change the global default settings, select **Protocol|Options** or click the Options toolbar button. Click the **Report** or **Export** tab as appropriate.



- Select the desired **Report** and/or **Export Engines,** and modify other parameters as necessary.

Important! If you change the engine *after* a document has been created for the protocol, the current document will be discarded and a new one must be created:

- Click **OK** to approve the selection of a new report or export engine. The current document (if one exists) will be discarded.
- Click **Cancel** to keep the previous engine and retain the current document (if one exists).

- See "Protocol Options" at the end of *Protocol Menu* for more information on the different Report and Result Export options.

Defining the Protocol Parameters

It is a good idea to define all or at least the majority of the protocol parameters *before* creating a report or export document. KC4 customizes its lists of options that can be inserted into the document based on the current protocol parameters.

For example:

- Define Standard wells in the Plate Layout dialog and set the curve-fitting parameters if the report will contain information about the **standard curve** (such as a picture of the curve, the curve equation, and the R/R^2 values).
- Define Sample wells in the Plate Layout dialog and specify cutoff criteria if the report will contain **custom formulas** to determine the frequency of symbols assigned to samples and then generate a pie chart showing the percentages.
- Define the wavelengths in the Reading parameters dialog and assign blank wells in the Plate Layout dialog if the export file will contain **raw and blanked measurement data** at 405 nm and 490 nm.

Creating/Editing Documents

When you are ready to create or edit a document, select **Protocol|Report** or **Protocol|Export,** or click the appropriate toolbar button:

Create/edit an Excel **report** document.

Create/edit an Excel **export** document.

Create/edit a Word **report** document.

Create/edit a Word **export** document.

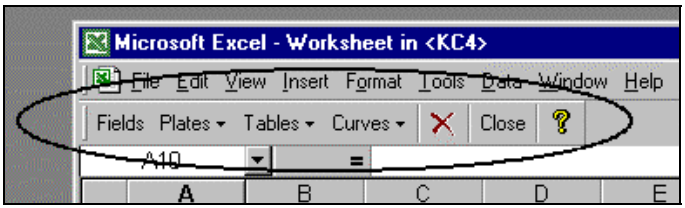
Tip: If you don't see these buttons in KC4's toolbar, you can add them by selecting **System|Toolbar.**

Working with Results Objects

A **Results Object** is a placeholder of information in an Excel or Word document that is filled in with results data during the print preview, print, or export function.

The Results Objects Toolbar

When KC4 launches Excel or Word, it automatically adds a **Result Objects** toolbar to the standard Excel/Word toolbar:


Microsoft Excel - Worksheet in <KC4>
File Edit View Insert Format Tools Data Window Help
Fields Plates Tables Curves Close
A10
A B C D E

Remember that a results object is a *placeholder* of information which will be filled in by KC4 after the plate is read. Each object specifies which item of information to use, where the information will be located in the document, and how it will be presented (font style, color, size, etc.).

Insert a **Field Object.** Each object contains a single piece of information, such as a cut-off value, a file name, or a curve coefficient. Clicking the Fields button opens a dialog with lists of available categories and related items to choose from. Categories include Curve Fit, Cut-Offs, File Information, Plate Description, and Reading Parameters.

Insert a **Plate Object** to present data in a row x column format matching the plate type defined in the protocol. Clicking the Plates button opens a list of available items to include in the plate, such as Well ID, 405, 405 Corr., and Concentrations.

Insert a **Table Object** to present data in a columnar format. Clicking the Tables button opens a list of available items to present in this format, including Statistics, Well Data Analysis, and Fitting Results.

Insert a **Curve Object,** such as a standard curve, a set of dose response curves, or a kinetic, scanning, or spectrum well zoom.

Delete an object. The cursor must be placed inside the object when this button is clicked.

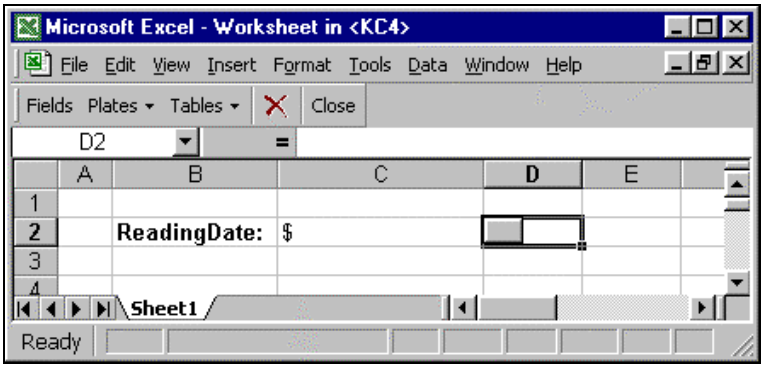
Close (minimize) Excel or Word and return to KC4. The document is automatically saved as a part of the protocol.

Get **Help** on PowerReports.

Object Components

Objects are inserted into the document using the Result Objects toolbar.
See *Field Objects*, *Plate Objects*, *Table Objects*, or *Curve Objects* for specific instructions for inserting the different types of objects.

The example below shows a **Field Object** in an Excel document:

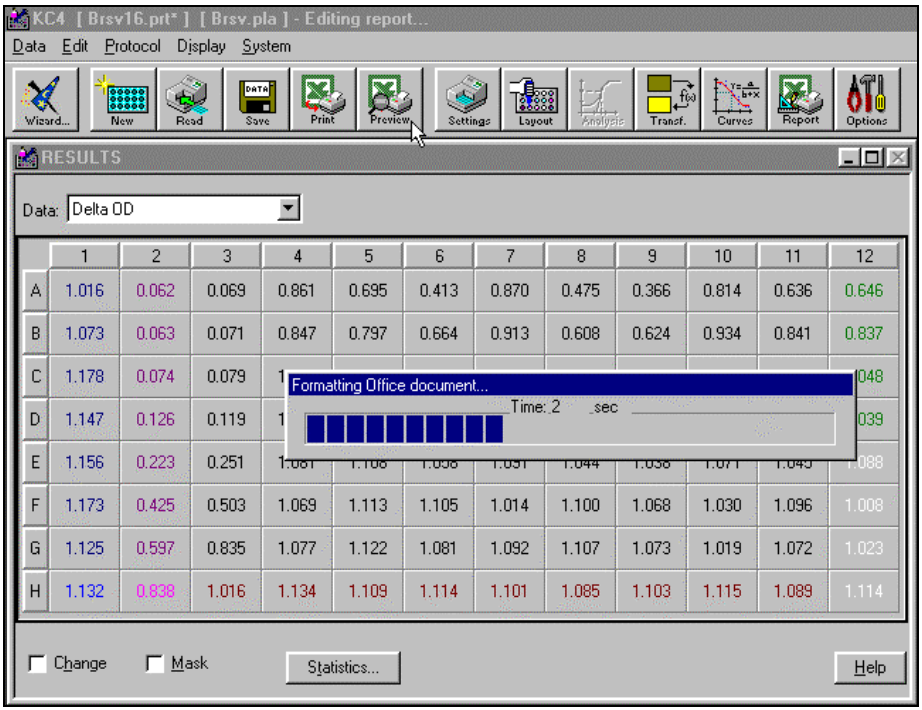


In the report or export document, any object contains 3 unique items:

- **An identifying title,** as shown in cell **B2.**
  - The text and appearance of this title can be modified. To change the text, simply overwrite the existing title. To change the appearance, use the application's font customization tools.
- **A dollar sign ($) character,** as shown in cell **C2.**
  - The dollar sign will be replaced by actual information during the print, print preview, or export process. In the example above, the dollar sign will be replaced by the system date recorded when the plate was read.
  - By default, the font color of this character is set to 'Auto'. The color will be whatever the color is in KC4. The color (and font type and size) can be overridden in the template using the application's font customization tools.
  - Do not delete or move a dollar sign without deleting or moving the entire object.
- **A gray editing button,** as shown in cell **D2.**
  - To change the contents of an existing object, double-click the gray editing button. The subsequent dialog depends on the object type.

Object Fulfillment

After a plate is read and the operator initiates the print preview, print, or export function KC4 begins the formatting process and transfers data into the prepared document. A **Formatting Office document…** message lets you know that something is happening:


KC4 [Brsv16.prt*] [Brsv.pla] - Editing report...
Data Edit Protocol Display System
Wizard... New Read Save Print Preview Settings Layout Analysis Transf. Curves Report Options
RESULTS
Data: Delta OD
Formatting Office document...
Time: 2 sec
Change Mask Statistics... Help

When formatting is complete, the document can be printed, viewed, or exported depending on the current process.

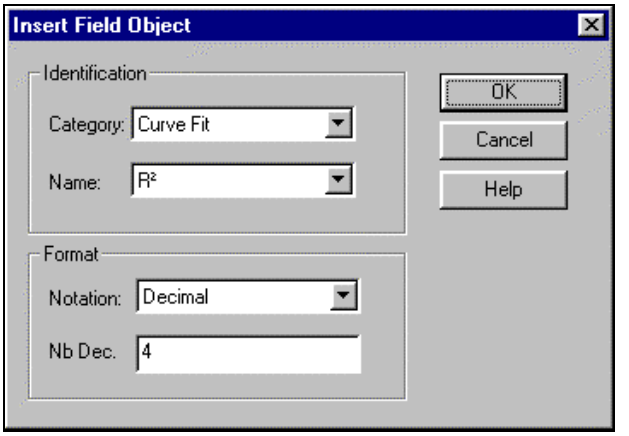
Designing the Document

This section explains how to work with Field, Plate, Table, and Curve Objects.

Inserting Field Objects

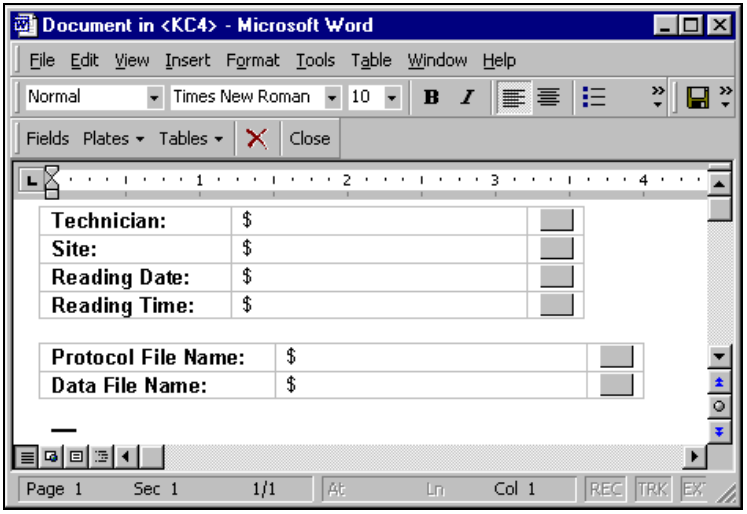
A **Field Object** represents a single item of information, such as the name of the protocol file, the calculated value of a cut-off, or the r-squared value of a curve.

To insert a Field Object, click the **Fields** button in the RSO toolbar. The **Insert Field Object** dialog appears:



- Select the **Category** of information that represents this Field Object. Available options include Curve Fit, Cut-Offs, File Information, Plate Description, Reading Parameters, and System Information.
- Select the **Name** of the field within that category.
  - If the only available item in the list is '*****' the current protocol parameters do not generate any fields for this category. For example, if the protocol does not specify cut-off criteria there will be no items available under the Cut-Offs category.
  - **Tip:** To learn more about the different fields and to see some examples, click on the Insert Field Object **Help** button then click on a Category link.
- If the field will contain a numeric value, set the **Notation** to Decimal (ex. 0.003) or Scientific (ex. 3E-03).
- If Decimal notation is selected, specify the number of decimal places (**Nb Dec**) to be displayed, 0 to 5.
- Click **OK** to close this dialog and insert the Field Object into the document.

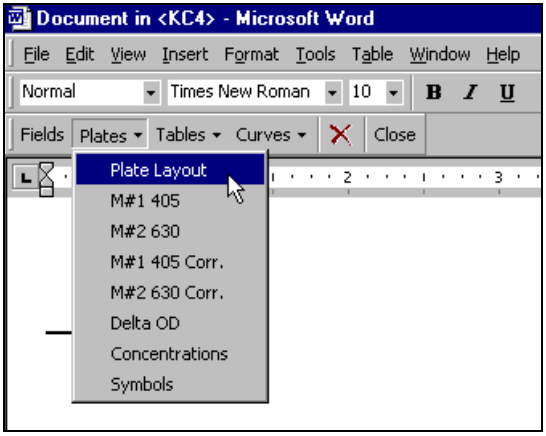
The document shown on the next page contains six Field Objects. Each object is represented by a 1 row x 3 column table. Notice that Field objects do not have to be separated by a blank line or row. See "Object Components" for more information.


Document in <KC4> - Microsoft Word
File Edit View Insert Format Tools Table Window Help
Normal Times New Roman 10
Fields Plates Tables Close
Technician: $
Site: $
Reading Date: $
Reading Time: $
Protocol File Name: $
Data File Name: $
Page 1 Sec 1 1/1 Col 1 REC TRK EXT

Inserting Plate Objects

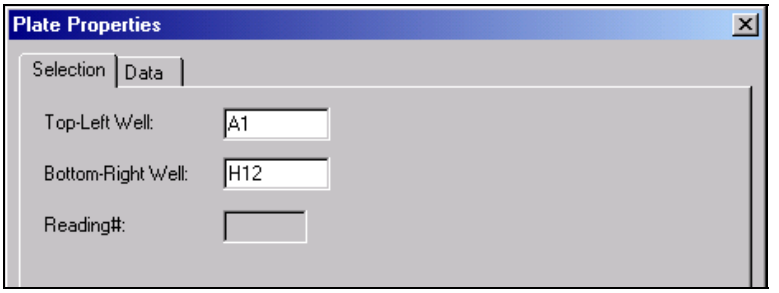
A **Plate Object** presents information in a matrix corresponding with the plate type selected in the protocol. For example, if the protocol specifies a 96-well plate, the plate object contains 8 rows and 12 columns of data.

To insert a Plate Object, click the **Plate** button in the RSO toolbar. A drop-down list appears, containing all of the data sets available for inclusion in the Plate Object:



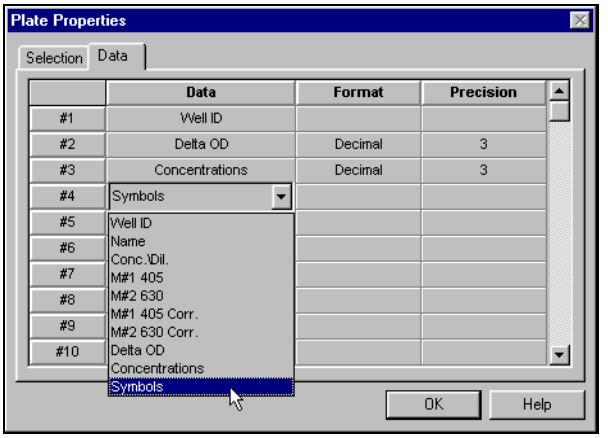
- Data set availability is based on the current protocol settings.
- Each object can contain from 1 to 20 data sets. For example, an object can contain the Well ID, the corrected data value, and the concentration value for every well. Or, it can contain just the concentrations.
- To proceed, select one data set (even if you want to include multiple data sets in the same object).

The **Plate Properties** dialog appears:



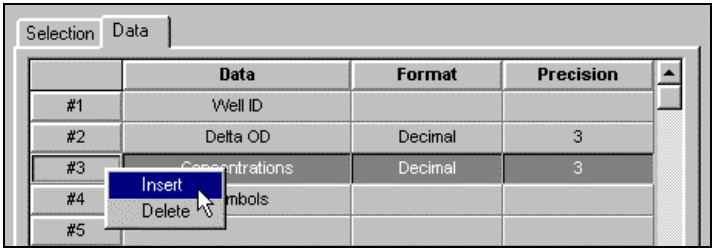
- The **Top-Left Well** and **Bottom-Right Well** set the boundaries for the plate object. The default settings coordinate with the plate type defined in the protocol.
- The **Reading #** field is available for kinetic protocols to specify which kinetic read to include in the object. Each plate object can display information for *one* kinetic read.

- Click the **Data** tab to select the data sets to include in the plate object. KC4 presents one or more default **Data** sets for inclusion in the object, although any available data set(s) can be selected.



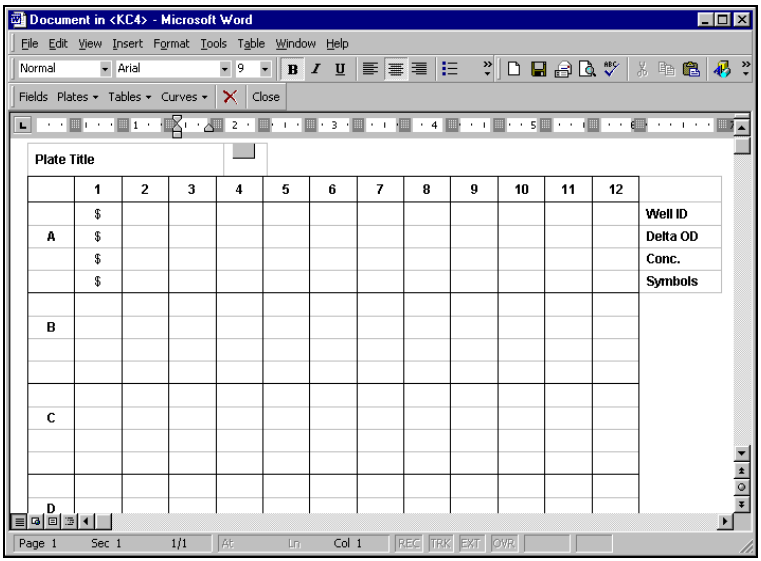
- To **add** a data set, highlight the first empty Data field and click the left mouse button once. A list of available data sets will appear; click on the desired set to select it.
- To **change** an existing data set, highlight it and click the left mouse button once. A list of available data sets will appear; click on the desired set to select it.
- To **delete** an existing data set, highlight its entire row then click the right mouse button and select **Delete.**

- To **insert** a new data set between two existing sets, highlight the row *above which* the new data set will be inserted. Click the right mouse button and select **Insert.**



For data sets that represent numerical information, click the corresponding **Format** field then select Decimal or Scientific. For the Decimal format, click the corresponding **Precision** field to set the number of decimal places (0 to 5).

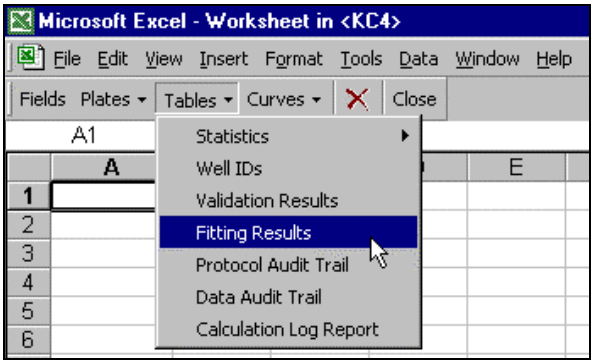
- Click **OK** to save the settings and insert the new Plate object into the document, as demonstrated below:



Inserting Table Objects

A **Table Object** presents results data in a columnar format.

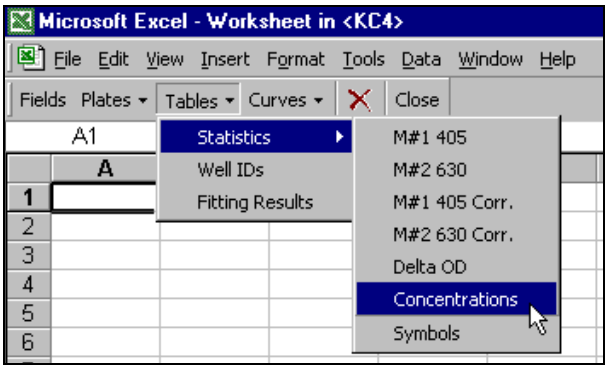
To insert a Table object, click the **Table** button in the RSO toolbar. A drop-down list of available data categories appears:



- This list contains all available categories of data, based on the **current protocol settings.**
- Available categories can include Statistics, Well IDs, Well Data, Well Analysis Results, Validation Results, Fitting Results, Protocol/Data Audit Trail, and Calculation Log Report.

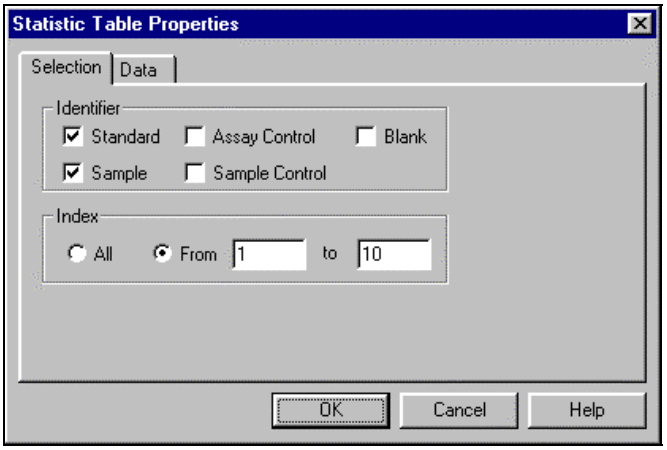
Statistics

A **Statistics table** calculates the Mean, Standard Deviation, and Coefficient of Variation (CV%) for the Standard, Sample, Control, and/or Blank groups defined in the protocol. To create a Statistics table, click the **Tables** button in the RSO toolbar, then highlight **Statistics.** A list of available data sets appears:



- The list contains those data sets made available by the current protocol parameters.
- Select the data set to be represented in the table.

The **Statistic Table Properties** dialog appears:

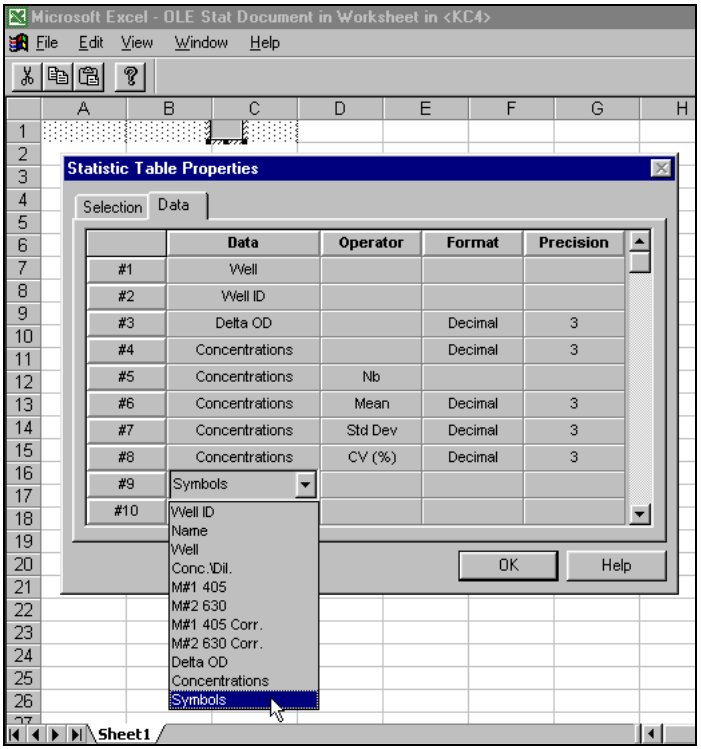


- Select the **Identifier(s)** to be represented in the table.

  If Sample and/or Assay Control is selected, **Index** is by default set to **All,** meaning all sample and/or assay control wells will be included in the table.

  To include a *subset* of all sample and/or assay control wells, use the Index **From** and **To** fields to enter the limiting group numbers. In the example above, Sample groups 1 through 10 (SPL1 to SPL10) will be represented in the table.

- Click the **Data** tab to customize the contents of the table.



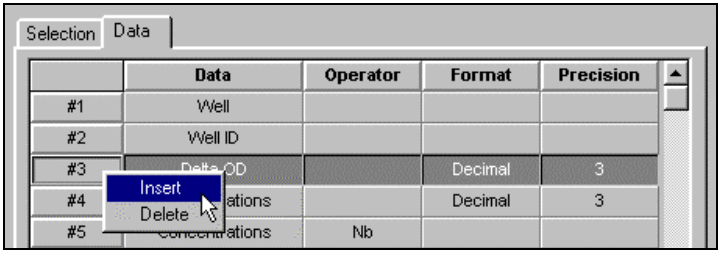
- KC4 presents some default **Data sets** for inclusion in the table, although any available data set(s) can be selected.

  To **add** a data set, highlight the first empty Data field and click the left mouse button once. A list of available data sets will appear; click on the desired set to select it.

  To **change** an existing data set, highlight it and click the left mouse button once. A list of available data sets will appear; click on the desired data set to select it.

  To **delete** an existing data set, highlight the entire row then click the right mouse button and select **Delete.**

  To **insert** a new data set between two existing sets, highlight the row *above which* the new data set will be inserted. Click the right mouse button and select **Insert.**



- For data sets that represent numerical information, an **Operator** can be applied to perform a specific calculation.

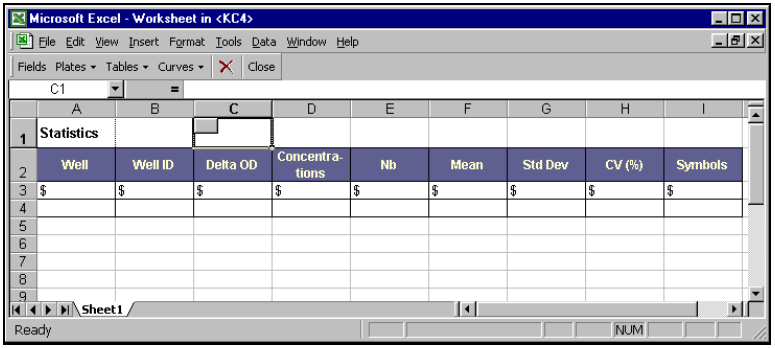
  Nb displays the number of wells in the group.

  Mean calculates the average measurement for each well group.

  Std Dev calculates the Standard Deviation for each well group.

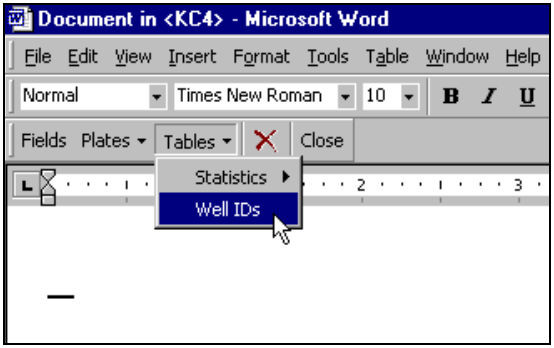
  CV (%) calculates the Coefficient of Variation for each well group as (Std Dev / Mean) x 100.

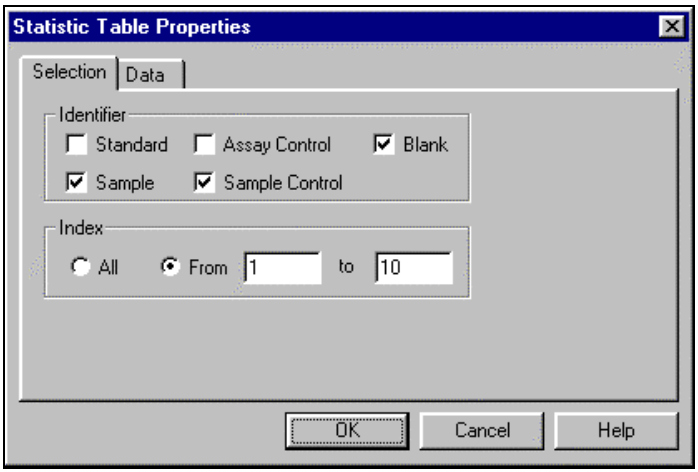
- Click **OK** to close this dialog and insert the object into the document.



Well IDs

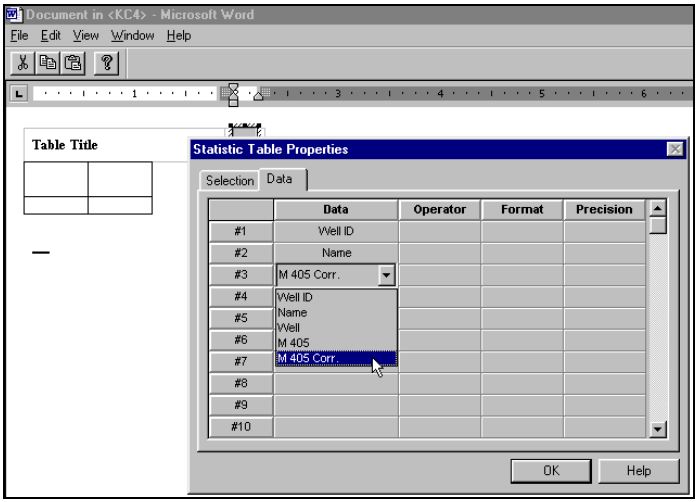
A Well IDs Table lists well identifiers and their names. To create a Well IDs table, click the **Tables** button in the RSO toolbar and then click on **Well IDs.** The **Statistic Table Properties** dialog appears.



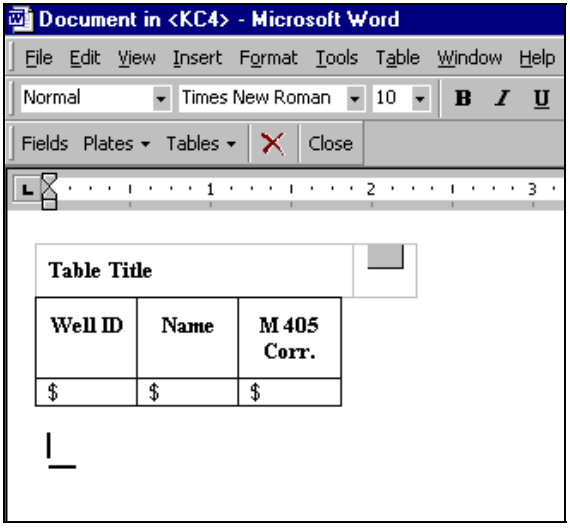


- Select the **Identifier(s)** to be represented in the table.
- If Sample and/or Assay Control is selected, **Index** is by default set to **All,** meaning all sample and/or assay control wells will be included in the table.
  - To include a *subset* of all sample and/or assay control wells, use the Index **From** and **To** fields to enter the limiting group numbers. In the example above, Sample groups 1 through 10 (SPL1 to SPL10) will be included in the table.

- Click the **Data** tab to customize the contents of the table.



  - For the Well IDs table, KC4 automatically inserts the **Well ID** and **Name** data sets.
  - Any available data set can be included in the list. See "Statistics" for information on working with data sets.
  - Click **OK** to close this dialog and insert the object into the document.

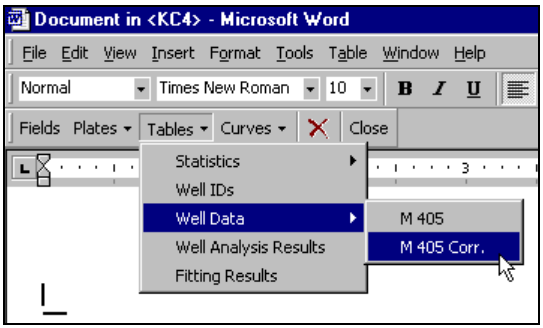


Well Data

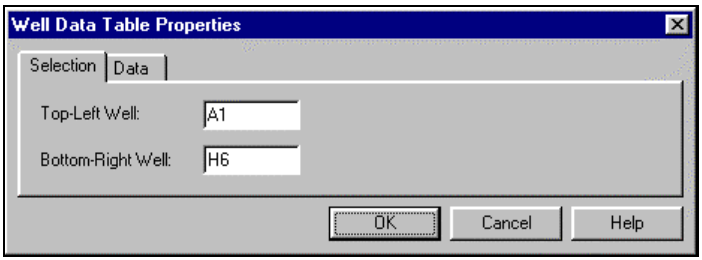
A **Well Data table** presents measurement values for kinetic, spectrum, or scanning protocols.

- For **Kinetic** protocols, the table lists measurement values by time of kinetic reading
- For **Spectrum** protocols, the table lists measurement values by wavelength
- For **Linear Scanning** protocols, the table lists measurement values by reading position
- For **Area Scanning** protocols, each table presents one well's data in a matrix format

To create a Well Data table, click the **Tables** button in the RSO toolbar and then highlight **Well Data** to open a list of available data sets. Click on the data set to be represented in the table; the Well Data Table Properties dialog appears.

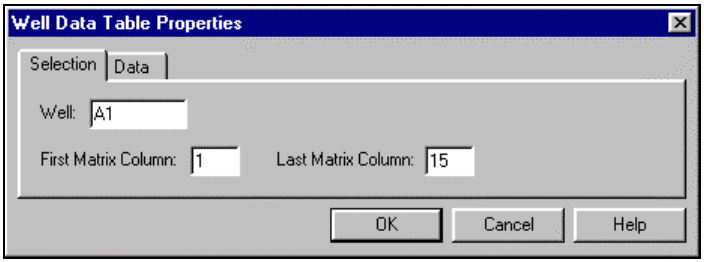


Kinetic, Spectrum, and Linear Scanning protocols:



- Define a **rectangle of wells** to be included in this table. Note: This table reports information in columns, one column per well. You may wish to experiment with the maximum number of columns that can display legibly per table. If you want to display information for many wells (i.e. more than 10-12), consider inserting multiple Well Data Table objects.

Area Scanning protocols:



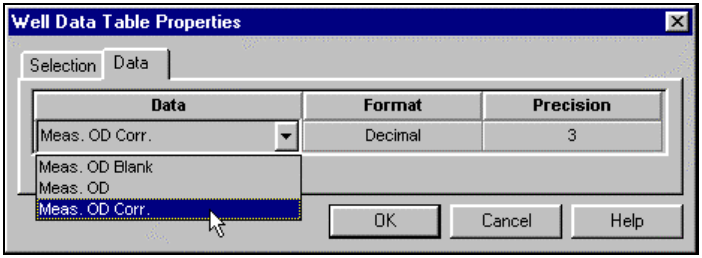
- Each table reports the area scanning data for one well. Specify the **Well** to be represented in the table.

- Area scanning data is reported in a matrix format, representing the measurement points taken across and down each well. Specify the **First** and **Last Matrix Column** to include in this table object.

  Note: If Word is the reporting/exporting engine, each table object can contain a maximum of 15 columns, so you may need to create multiple table objects to print all of the information for the well. For example, if the Read Matrix Size is 25 x 25, create one table object with First and Last Matrix Columns set to 1 and 15, then create another table object with First and Last Matrix Columns set to 16 and 25.

All protocols:

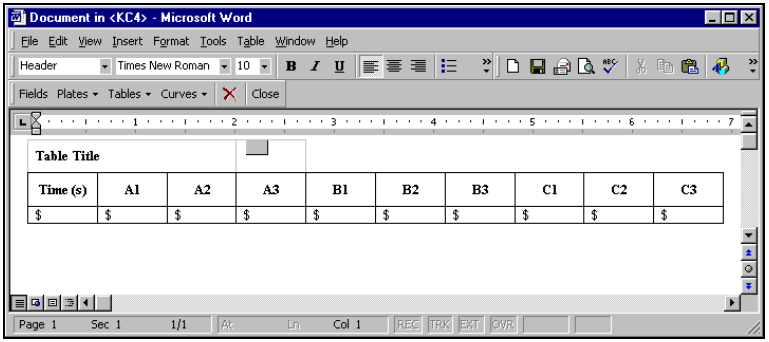
- Click the **Data** tab to change and/or customize the data set.



  - Each Well Data table reports information for *one* data set. If the current protocol parameters generate multiple data sets, click on the **Data** field to open a drop-down list of all available data sets.

    Click the **Format** field then select Decimal or Scientific. Click the **Precision** field to set the number of decimal places (0 to 5).

  - Click **OK** to close this dialog and insert the object into the document.

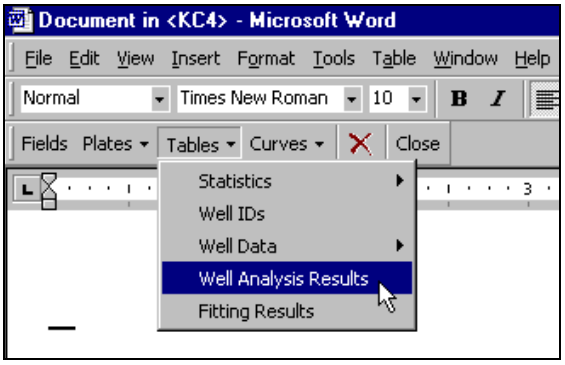


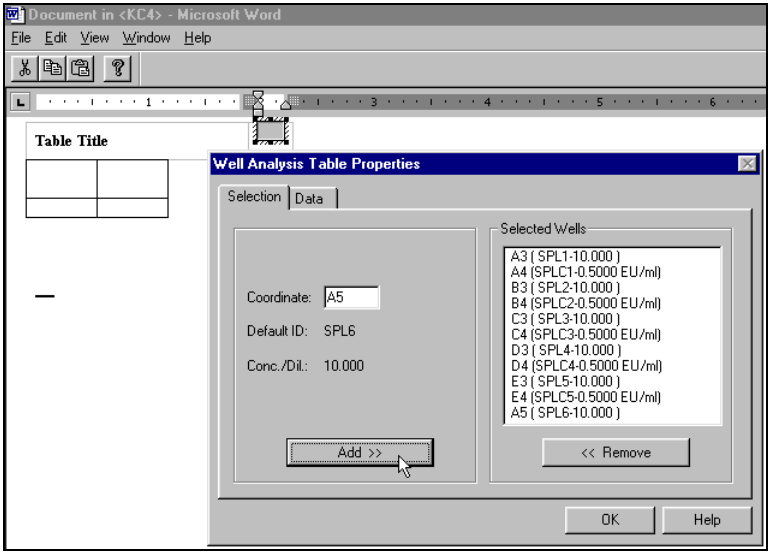
Well Analysis Results

A **Well Analysis Results** table lists various statistics for kinetic, spectrum, or scanning protocols.

- For **Kinetic** protocols, the table lists the kinetic analysis calculations (e.g. Max V, Delta t at Max V, Lag Time, etc.), determined for each well.
- For **Spectrum** protocols, the table lists the Minimum and Maximum measurements and their corresponding wavelength values for each well.
- For **Area** and **Linear Scanning** protocols, the table lists the Mean OD, Standard Deviation, and Coefficient of Variation calculated for each well.

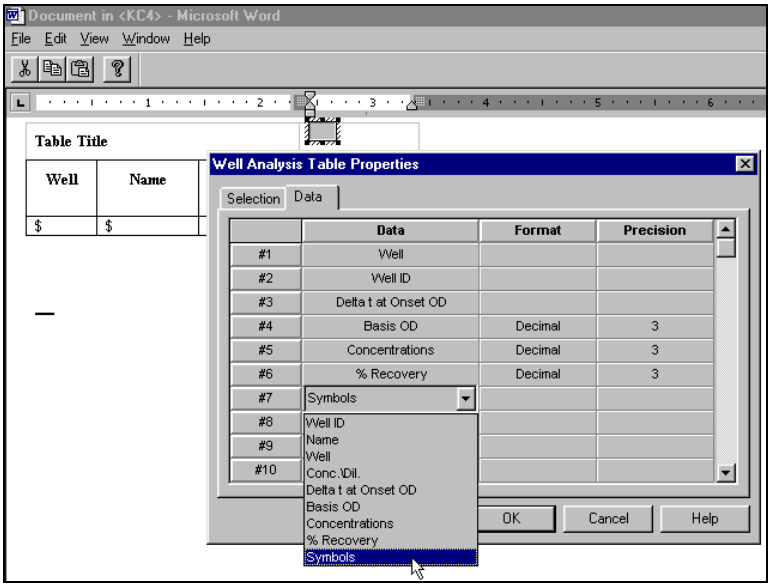
To create a Well Analysis Results table, click the **Tables** button in the RSO toolbar and then click on **Well Analysis Results.** The **Well Analysis Table Properties** dialog appears:



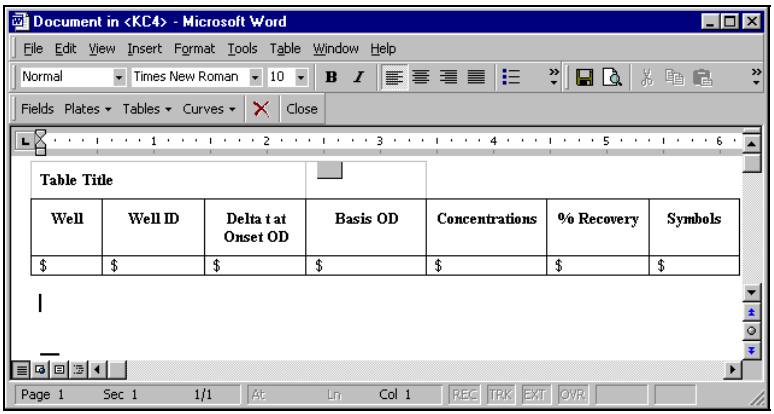


- The **Selected Wells** list contains all wells that will be included in the table.
  - To append a well to the Selected Wells list, enter the well's **Coordinate** in a format resembling A1 or F11, then click the **Add>>** button.
  - To remove a well from the Selected Wells list, highlight the well then click **<<Remove.**

- Click the **Data** tab to customize the information to be included in the table for the selected wells.



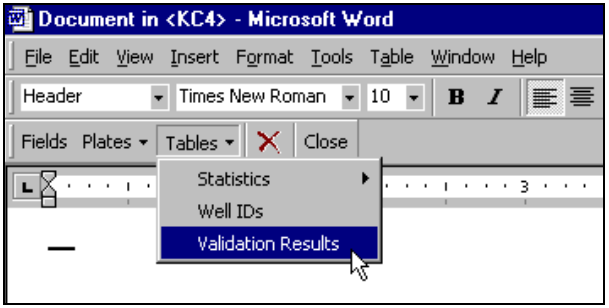
  - KC4 presents a default list of **Data** sets to be included in the table, although any available data set can be selected. See "Statistics" for information on working with data sets.
  - Click **OK** to close this dialog and insert the object into the document.

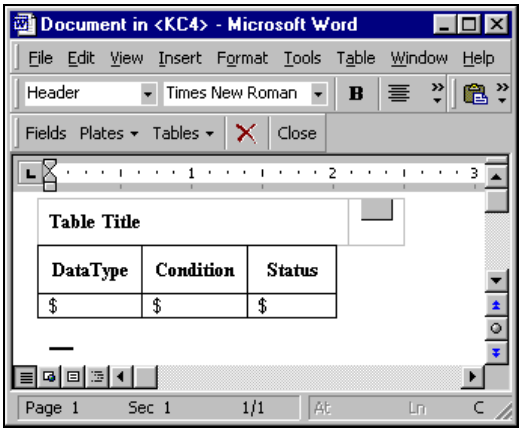


Validation Results

A **Validation Results** table lists the validation conditions and their corresponding statuses.

To create a Validation Results table, click the **Tables** button in the RSO toolbar, then click on **Validation Results.** The table object is automatically inserted into the document.

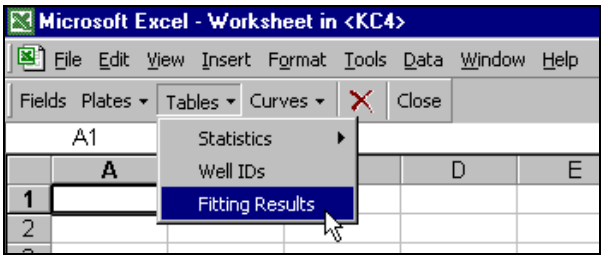


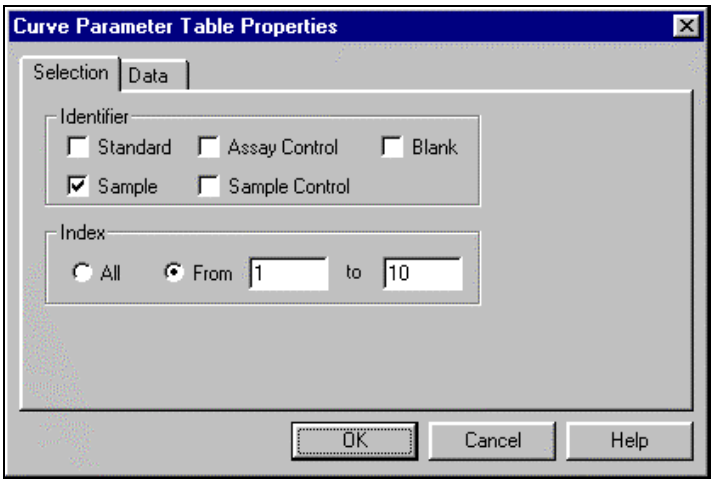


Fitting Results

A **Fitting Results** table lists coefficient values for all curves in the assay.

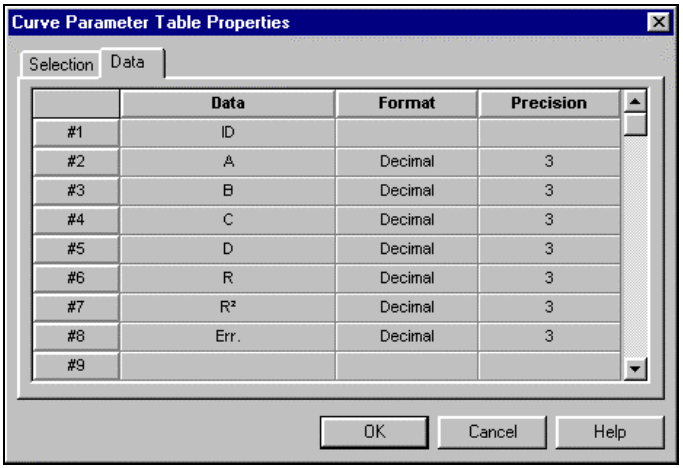
To create a Fitting Results table, click the **Tables** button in the RSO toolbar, and then click on **Fitting Results.** The **Curve Parameter Table Properties** dialog appears.





- Select the **Identifier(s)** to be represented in the table.
  - If Sample and/or Assay Control is selected, **Index** is by default set to **All,** meaning all sample and/or assay control wells will be included in the table.
  - To include a *subset* of all sample and/or assay control wells, use the Index **From** and **To** fields to enter the limiting group numbers. In the example above, Sample groups 1 and 2 (SPL1 and SPL2), will be included in the table.

- Click the **Data** tab to customize the contents of the table.



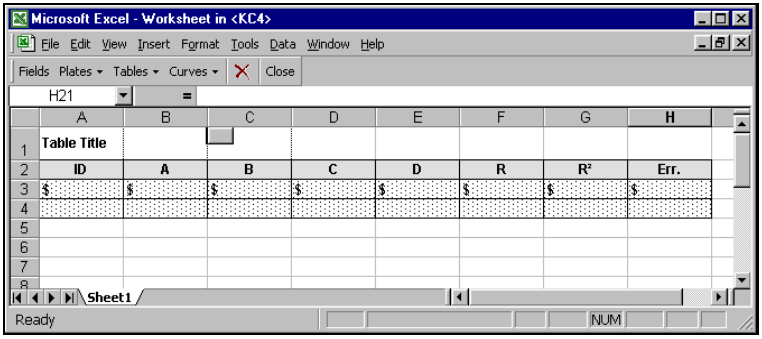
KC4 presents a default set of **Data** items that will be included in the table, although any available item can be included in the list.

- To **add** an item to the list, highlight the first empty Data field and click the left mouse button once. A list of available data items will appear. Click on the desired item.
- To **modify** an item in the list, highlight it and click the left mouse button once. A list of available data items will appear. Click on the desired item.
- To **delete** an item from the list, highlight the row then click the right mouse button and select **Delete.**
- To **insert** a new item between two existing items, highlight the row above which the new item will be inserted. Click the right mouse button and select **Insert.**

For data sets that represent numerical information:

- Click the corresponding **Format** field then select Decimal or Scientific.
- For the Decimal format, click the corresponding **Precision** field to set the number of decimal places (0 to 5).

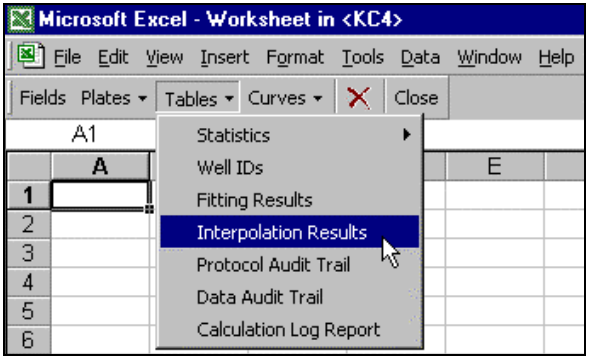
Click **OK** to close this dialog and insert the object into the document.

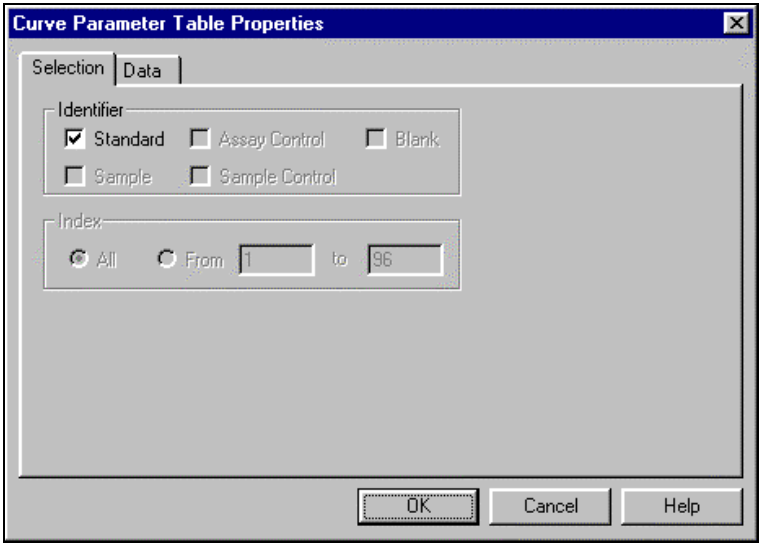


Interpolation Results

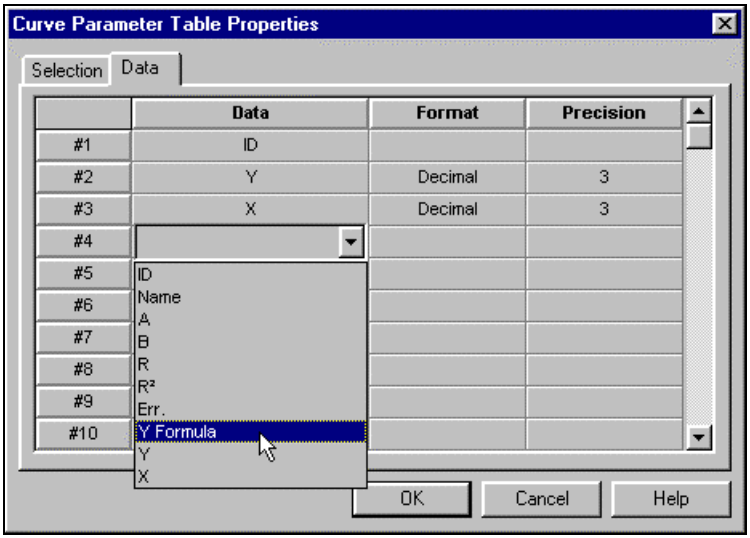
An **Interpolation Results** table lists the calculated X-values for the Y-values and formulas entered under Protocol|Curves|Interpolations.

To create an Interpolation Results table, click the **Tables** button in the RSO toolbar, and then click on **Interpolation Results.** The **Curve Parameter Table Properties** dialog appears:



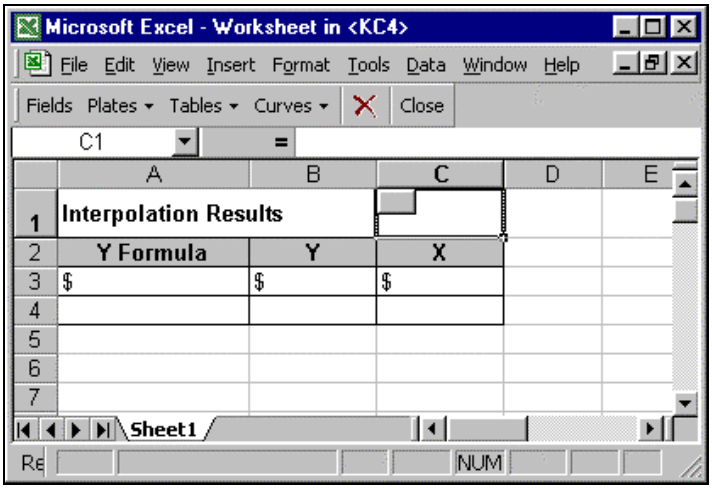


- *Standard* is the only **Identifier** that can be included in an Interpolation Results report.
- Click the **Data** tab to customize the contents of the Interpolation Results table.



KC4 presents a default set of **Data** items that will be included in the table, although any available item can be included in the list. See the previous topic, "Fitting Results" for information on working with Data items.

Click **OK** to close this dialog and insert the object into the document.

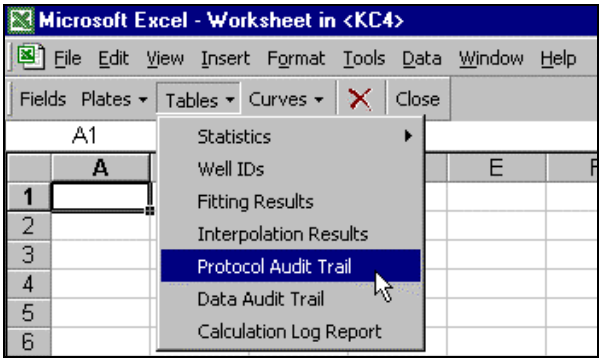


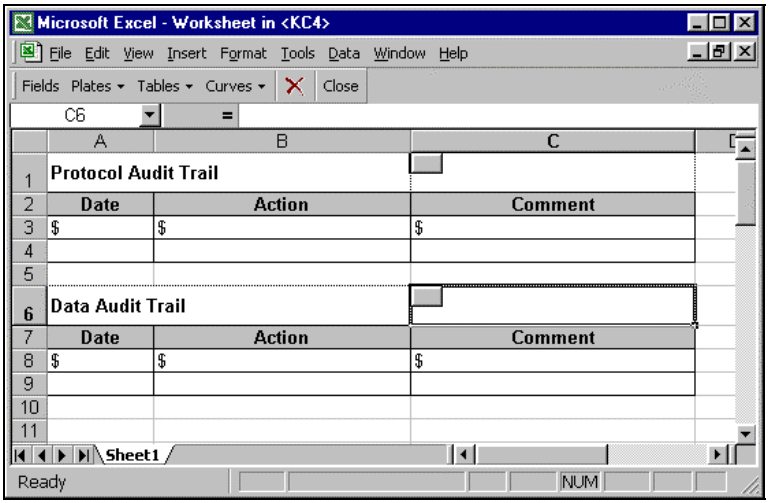
Protocol/Data Audit Trail

Protocol and **Data Audit Trail** information can be included on reports and in export files.

Note: The Protocol Audit Trail is only available if the Preferred Data File Format setting is PLA.

To create a Protocol or Data Audit Trail table, click the **Tables** button in the RSO toolbar then click on **Protocol Audit Trail** or **Data Audit Trail.** The table is automatically inserted into the document (the example below shows two separate tables).

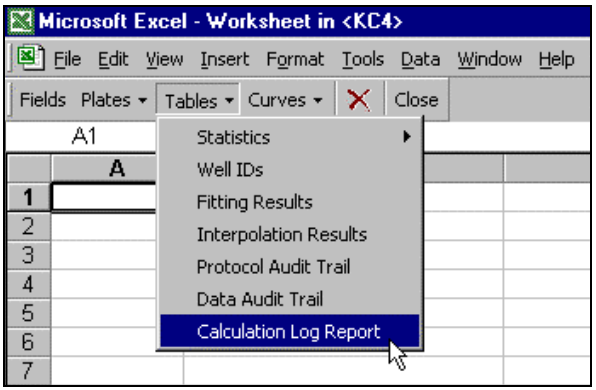


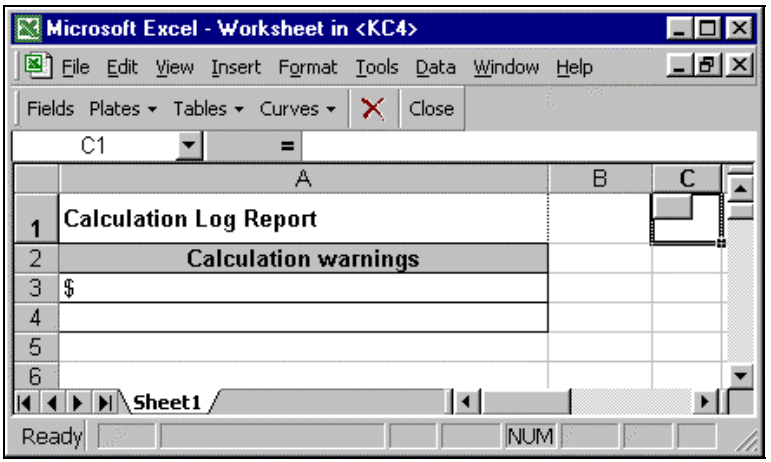


Calculation Log Report

A **Calculation Log Report** can be included on reports and in export files.

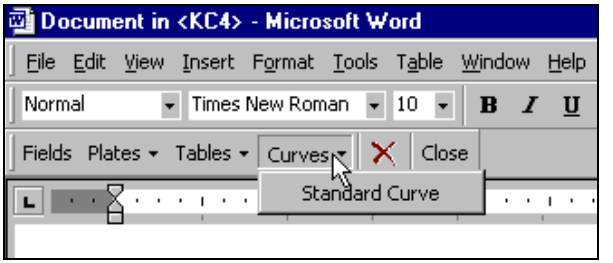
To create a Calculation Log Report table, click the **Tables** button in the RSO toolbar and then click on **Calculation Log Report.** The table is automatically inserted into the document:





Inserting Curve Objects

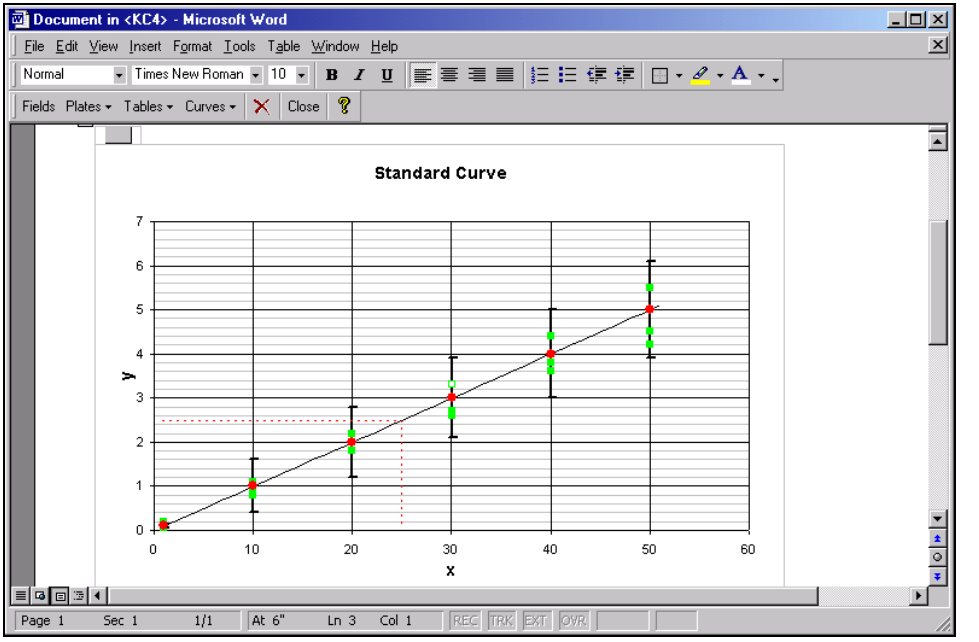
A **Curve Object** inserts a graph showing one or multiple overlaid curves. To insert a Curve Object, click the **Curves** button in the RSO toolbar. A drop-down list showing the available curve type will appear:



- This list contains all available curve types, based on the **current protocol settings.** Available curve types can include Standard, Dose Response, and Well Zoom.

Standard Curve

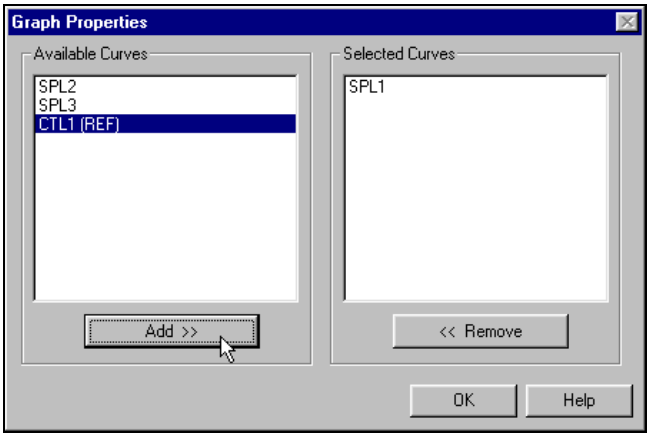
A standard curve is generated by a protocol that has Standard wells defined in the Plate Layout. To insert a Standard Curve, click on the **Curves** button in the RSO toolbar, then click on **Standard Curve** to insert the object.



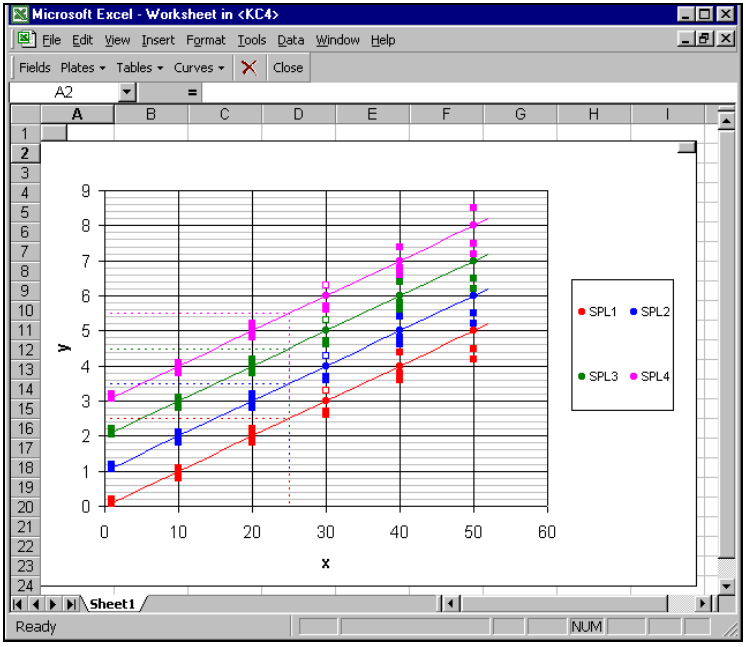
- KC4 generates a temporary curve using fictitious data. After the plate is read, KC4 rebuilds the curve with real plate data.
- Certain characteristics of this curve can be modified, including colors and labels. Double-click anywhere on the graph to enter the editing mode; a shaded box should appear around the graph. When this box appears, click the right mouse button once to open a menu of items that can be edited.

Dose-Response Curve(s)

Dose response curves are generated by a protocol with assay control, sample, or sample control wells with concentrations or increasing dilutions defined. Up to 5 curves can be overlaid in one curve object. To insert a Dose Response curve, click on the **Curves** button in the RSO toolbar, then click on **Dose Response.** The **Graph Properties** dialog appears, listing all available curves by Well ID:



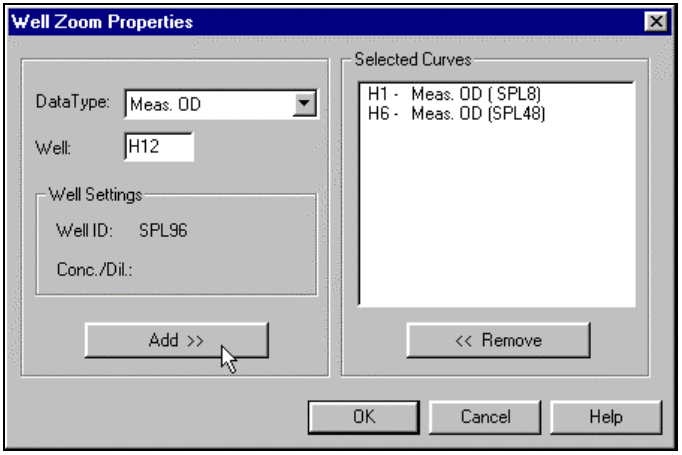
- The **Available Curves** box lists all curves made available by the current protocol parameters.
- The **Selected Curves** box lists all curves that will be overlaid in a single curve object.
- To add a curve to the list, highlight an available curve, then click **Add >>.**
- To remove a curve from the list, highlight the curve, then click **<< Remove.**
- Click **OK** to close the dialog and insert the object into the document.



- KC4 generates temporary curve(s) using fictitious data. After the plate is read, KC4 rebuilds the curve(s) with real plate data.
- Certain characteristics of this curve can be modified, including colors and labels. Double-click anywhere on the graph to enter the editing mode; a shaded box should appear around the graph. When this box appears, click the right mouse button once to open a menu of items that can be edited.

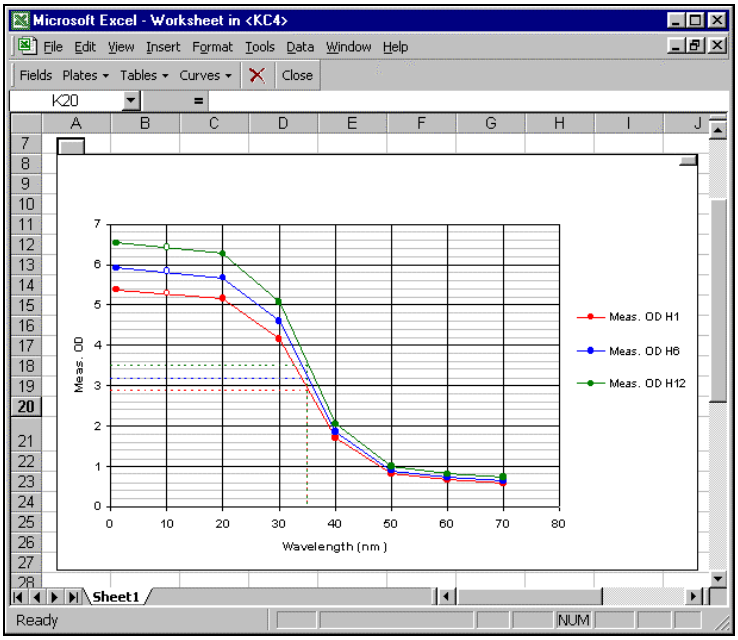
Well Zoom Curve(s)

Well zoom curves are available for kinetic, spectrum, and scanning protocols. Up to five curves can be overlaid in one object. To insert a Well Zoom curve object, click on the **Curves** button in the RSO toolbar, then click on **Well Zoom.** The **Well Zoom Properties** dialog appears:



The **Selected Curves** box lists all wells that will be overlaid in the curve object.

- To add an item to the Selected Curves list:
  - Choose a **Data Type** from the drop-down list. The contents of this list depend on the current protocol parameters. Data types can represent raw, blank plate, and corrected data.
  - Enter the **Well** coordinates. The corresponding Well ID and Conc./Dil. value will appear, if applicable.
  - Click **Add >>.**
- To remove an item from the list, highlight the item then click **<< Remove.**
- Click **OK** to close the dialog and insert the object into the document.



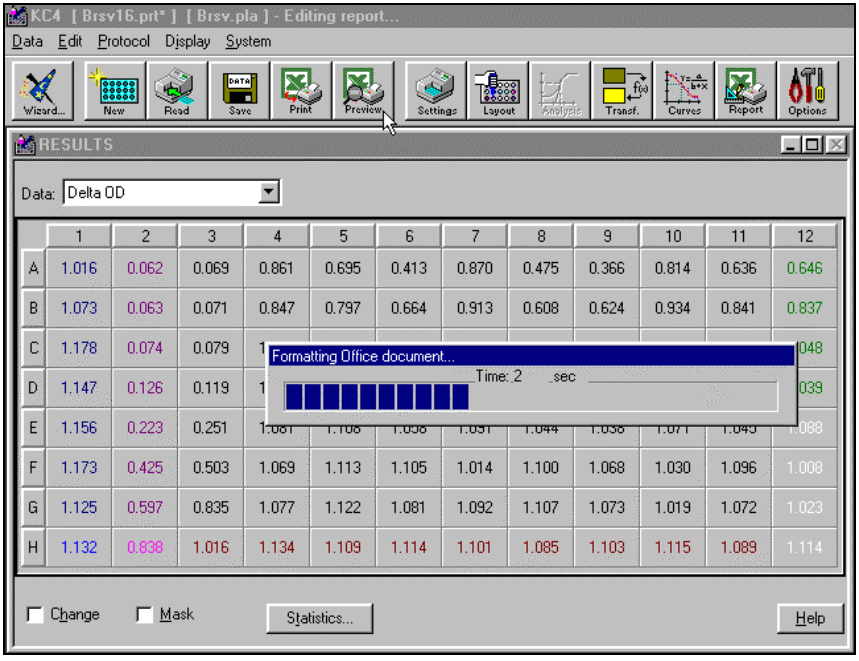
- KC4 generates temporary curve(s) using fictitious data. After the plate is read, KC4 rebuilds the curve(s) with real plate data.
- Certain characteristics of this curve can be modified, including colors and labels. Double-click anywhere on the graph to enter the editing mode; a shaded box should appear around the graph. When this box appears, click the right mouse button once to open a menu of items that can be edited.

Previewing and Printing the Report

When plate reading and data reduction are complete, the report is ready for formatting and then previewing and/or printing.

To format the report (that is, to fill all of the objects in with current plate data), select **Data|Print Preview** or **Data|Print,** or click the appropriate toolbar button.

During the formatting process, KC4 displays a progress bar, as shown below:



Previewing Options

When preparing to print a newly created or recently edited report, it's a good idea to preview it first to check column widths, page breaks, fonts, colors, and so on.

If a change is required before printing, two options are available:

- Modify the report document and go through the print preview process again. This is the recommended option because changes will be saved with the protocol.
- Make a temporary change in the preview document. The Protocol|Options parameter **Keep Open After Printing** must be enabled.

Special Notations

When displaying results or when reporting results using the standard (KC4) reporting engine, KC4 uses various font characteristics to denote masked wells and biased values.

When exporting results or when reporting results using the Excel and Word reporting engines, KC4 uses some special characters to denote masked wells and biased values. This is to avoid conflict with any special font characteristics that may be assigned by the report/export file designer.

- To denote a masked well, KC4 surrounds the value with asterisks: *0.234*, *?????*, *98999*.
- To denote biased values, KC4 surrounds the value with exclamation points: !0.068!, !23.456!.

Note: Biased values may appear in a Statistics table if **Use out of range values in statistics** is enabled under System|Setup. If, for example, the concentration for one of three standard replicates is reported as '>40', the Standard Deviation and CV% values are considered suspect. KC4 surrounds the values with exclamation points to indicate that it is the user's responsibility to determine whether or not the results are valid.

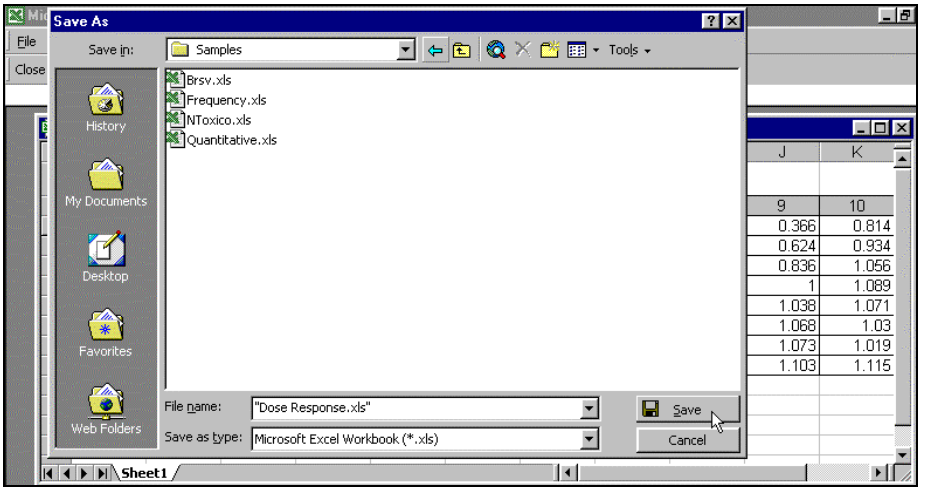
Statistics

Well ID	Conc.\Dil.	Well	M 405	Concentrations	Nb	Mean	Std Dev	CV (%)
STD1	10	A1	0.006	9.024	3	9.026	0.004	0.042
		A2	0.007	9.032				
		A3	0.006	9.024				
STD2	20	B1	1.235	18.982	3	18.955	0.02	0.107
		B2	1.229	18.934				
		B3	1.231	18.95				
STD3	30	C1	4.999	>41.500	3	>32.350	!6.470!	!20.001!
		C2	2.319	27.766				
		C3	2.321	27.782				
STD4	40	D1	3.458	36.996	2	37.008	0.012	0.033
		D2	3.461	37.02				
		D3	*3.998*	*?????*				

Performing the Data Export

When plate reading and data reduction are complete, the export document is ready for formatting and saving.

To format the export document (that is, to fill all of the objects in with current plate data), select **Data|Export** or click the appropriate toolbar button. When formatting completes, KC4 displays the **Save As** dialog:



- Change the current folder and file name if desired, then click **Save** to complete the export.
- If the Protocol|Options parameter **Keep Open After Exporting** is enabled, the document stays open after the export is complete. This allows you to, for example, print the contents of the file.
- See "Special Notations" on the previous page for information on how KC4 denotes masked wells and biased values in export files.

Direct Export to Excel

KC4 provides a way to directly export data to Excel without first having to create an export template. To export plate data directly to Excel:

1. Under **Protocol|Options** (or under System|Setup if no protocol is open) set the export engine to Microsoft® Excel.
2. **Read** the plate.
3. Select **Data|Export.** KC4 will launch Excel and insert the RSO toolbar.
4. **Insert objects** (Field, Plate, Table, and/or Curve) as explained throughout this chapter.
5. Click **Save As** to export the data.

Appendix A: Records Submission

Records Submission

When preparing electronic records for submission to the FDA, contact Bio-Tek Instruments' Marketing & Sales Department for guidance, and to obtain a demonstration copy of KC4 to include with your package. The FDA will need KC4 to view and print your records.

Contact information:

Phone: (888) 451-5171 (toll free in the U.S.)
(802) 655-4740 (outside the U.S.)

Fax: (802) 655-7941

E-mail: customercare@biotek.com

Appendix B: Windows File Caching

Windows File Caching

This topic applies to sites accessing a shared database stored on a network/server.

By design, most write and read database operations are intended to be performed synchronously by KC4. For example, when a system audit trail event or a file is created by a KC4 instance, it should be immediately visible in other instances using the same database. However, because of other factors beyond the control of KC4, like network and operating system delayed-write optimizations, there is no way to guarantee such behavior. To improve synchronization, it is recommended, when KC4 is used on several networked PCs connected to the same shared database, to disable the network redirector file caching.

In addition, while file caching is enabled there is a *potential* for data loss following a network connection failure. According to Microsoft, when the Windows redirector opens a file for read or read/write access, the redirector utilizes the Windows system cache. Therefore, when data is written to the file, it is written to the cache and not immediately flushed to the redirector. The cache manager flushes the data at a later time. If an unrecoverable network error occurs while the data is being transferred to the remote server, it may cause the write request to fail and an application error to occur.

Here is an example scenario: The network connection failed because of a problem on the server. The KC4 user, unaware of the failure, chose to save a GLB file to the shared database just seconds *after* the failure. Because file caching was enabled, the file actually went into temporary storage in the Windows system cache, to await redirection to the shared database on the network. KC4 gave the appearance that the file was saved successfully (no error message appeared, and the asterisk disappeared from the title bar). After a moment, Windows detected the failure and displayed an appropriate error message, and the user decided to quit KC4. Upon closing, KC4 did not present an option to save the file because KC4 "thought" that the file was already saved. When the network connection was re-established, the user launched KC4 but could not locate the GLB file because it never reached the shared database.

For more information, visit http://support.microsoft.com and search for current articles on "file caching" or "write caching".

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